Mood and anxiety problems in young people with ASD
Focus on measurement and neurophysiological mechanisms

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Mood and anxiety problems in young people with ASD: Focus on measurement and neurophysiological mechanisms

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Abstract

Beyond core impairments in social interaction skills and restricted, repetitive behaviours, young people with autism spectrum disorder (ASD) often also suffer from debilitating mood and anxiety problems. The reasons for this overlap are poorly understood and often complicated by difficulties in symptom reporting in youth with ASD. This thesis used a multi-method approach to investigate two possible mechanisms underlying mood and anxiety problems in ASD youth, and examined the usefulness of neuroimaging methods (arterial spin labelling) to aid the detection of mood states without relying on clinical report.

We first investigated symptom reporting of irritability and physiological stress responses in boys with high-functioning ASD and typically-developing controls. Boys with ASD reported reliably on their irritability, and showed reduced cortisol and heart rate responsiveness to stress compared to controls. Cortisol hypo-responsiveness in boys with ASD was associated with irritability, while lower heart rate was associated predominantly with anxiety.

Second, we examined the interplay between ASD traits and anxiety during a neuroimaging reward paradigm in 1472 adolescents from the community. Shared and unique neural correlates of anxiety were found in those with high vs. low ASD traits; while specific brain activations during reward anticipation predicted future, new-onset anxiety in participants high on ASD traits. Symptoms of depression and irritability had minimal impact on the results.

Third, arterial spin labelling was sensitive to experimentally-induced mood states in healthy youth. It showed some usefulness for detecting specific mood states, whereby sad and happy moods were distinguished from neutral, but not from each other, based on brain activation patterns alone.

Our results suggest that some pathophysiological mechanisms of anxiety may be different in ASD youth compared to controls, bringing important clinical implications. The effects of anxiety on cortisol stress responsiveness, but not heart rate responsiveness or reward processing, may be better explained by co-occurring irritability.

Statement of work

This thesis used data from three independent studies.

Chapter 3 utilised data from the study "Assessment of anxiety in young people" led by Professor Emily Simonoff, funded by the South London and Maudsley (SLaM) Charitable Funds and supported by the Department of Health via the National Institute for Health Research (NIHR) Biomedical Research Centre (BRC) and Dementia Unit awarded to SLaM NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust. Additional data for this study were obtained from a dataset on severe mood dysregulation in collaboration with Dr Ellen Leibenluft from the National Institute of Mental Health in the US. Chapter 4 used data from the European Union-funded Sixth Framework Programme Integrated Project IMAGEN (ref. LSHM-CT-2007-037286); coordinated by Professor Gunter Schumann in the UK.

I was not involved in the planning, data collection or pre-processing of neuroimaging and physiological raw data for the above two studies. The research questions for studies in Chapters 3 and 4 were developed by myself with the guidance of Dr Argyris Stringaris and Professor Simonoff. I had responsibility for analysing and interpreting the data for studies in Chapters 3 and 4, and writing up of the results, under the supervision of Dr Stringaris and Professor Simonoff and with feedback from co-authors. Piecewise regression models presented in Chapter 3 (Section 0) were designed by Dr Mizanur Khondoker.

The third, arterial spin labelling project (Chapter 5) was founded by the NIHR Mental Health BRC EMCT Pilot Funding Award awarded to Dr Stringaris. I had sole responsibility for the design of the experimental paradigm, recruitment of participants and data collection. I was also responsible for data pre-processing, analysis, and writing up of the results under the supervision of and with feedback from the three co-authors: Drs Mitul Mehta, Fernando Zelaya, and Argyris Stringaris. I also contributed to the ethics and grant applications for this project. The MRI sequence used in this study was designed by Dr Zelaya.

The publication of findings resulting from this thesis is a direct product of my own work, achieved with the supervision from Dr Stringaris and Professor Simonoff and feedback from co-authors. When referring to the methods and results of individual studies included in this thesis, I use the third person ("we", "our") for consistency with published articles.

This thesis represents my own, original work.

List of publications and presentations relevant to this thesis

Publications

Parts of chapter 1 are adapted from:

Mikita, N., & Stringaris, A. (2013). Mood dysregulation. *European Child & Adolescent Psychiatry*, 22(Suppl 1), 11–16.

Chapter 3 is adapted from:

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Chapter 4 is adapted from:

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Presentations

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Abbreviations

ABC	Aberrant Behavior Checklist
ACC	anterior cingulate cortex
ACI-PL	Autism Comorbidity Interview-Present and Lifetime
ACTH	adrenocorticotropin hormone
ADHD	attention-deficit/hyperactivity disorder
ADI-R	Autism Diagnostic Interview-Revised
ADIS-C/P	Anxiety Disorders Interview Schedule: Child and Parent Versions
ADOS-G	Autism Diagnostic Observation Schedule-Generic
ALSPAC	Avon Longitudinal Study of Parents and Children
ANS	autonomic nervous system
APA	American Psychiatric Association
ARI	Affective Reactivity Index
ASD	autism spectrum disorder
ASL	arterial spin labelling
BA	Brodmann area
BAS	behavioural activation system
BDD	body dysmorphic disorder
BIS	behavioural inhibition system
BOLD	blood-oxygen-level dependent
CAPA	Child and Adolescent Psychiatric Assessment
CAR	cortisol awakening response
CBCL	Child Behavior Checklist
CBT	cognitive behaviour therapy
CD	conduct disorder
CGAS	Children's Global Assessment Scale
ChIPS	Children's Interview for Psychiatric Symptoms
CI	confidence interval
CRH	corticotropin releasing hormone
CU	callous-unemotional
CY-BOCS	Children’s Yale-Brown Obsessive-Compulsive Scale
dACC	dorsal anterior cingulate cortex
DASH-II	Diagnostic Assessment for the Severely Handicapped-II
dlPFC	dorsolateral prefrontal cortex
dmPFC	dorsomedial prefrontal cortex
ECG	electrocardiogram
EEG	electroencephalography

ERN	error-related negativity
ERP	event-related potentials
DAWBA	Development and Well-Being Assessment
DISC	Diagnostic Interview Schedule for Children
DMN	default mode network
DSM	Diagnostic and Statistical Manual of Mental Disorders
(f)MRI	(functional) magnetic resonance imaging
FWE	family-wise error
GAD	generalised anxiety disorder
GPC	Gaussian process classifier
HC	healthy controls
hfASD	high-functioning ASD
HPA	hypothalamic-pituitary-adrenal (axis)
HR	heart rate
HRV	heart rate variability
ICD	International Classification of Diseases
IWS	Isle of Wight Semistructured Informant Interview
IFG	inferior frontal gyrus
IQ	intelligence quotient
IPL	inferior parietal lobule
K-SADS	Kiddie Schedule for Affective Disorders and Schizophrenia
KID-SCID	Structured Clinical Interview for DSM-IV Childhood Diagnoses
LF/HF	low frequency / high frequency
LOOCV	leave one-out cross validation
MASC	Multidimensional Anxiety Scale for Children
MCC	midcingulate cortex
MDD	major depressive disorder
medFG	medial frontal gyrus
MFG	middle frontal gyrus
MFQ	Mood and Feelings Questionnaire
MID	monetary incentive delay
MIST	Montreal Imaging Stress Task
MNI	Montreal Neurological Institute
MTG	middle temporal gyrus
NAcc	nucleus accumbens
OCD	obsessive compulsive disorder
ODD	oppositional defiant disorder
OFC	orbitofrontal cortex

OR	odds ratio
PBS	Pediatric Behavior Scale
PCC	posterior cingulate cortex
PDD-NOS	pervasive developmental disorder-not otherwise specified
PEP	pre-ejection period
PET	positron emission tomography
PFC	prefrontal cortex
PONS	Profile of Neuropsychiatric Symptoms
PST	psychosocial stress test
PTSD	post-traumatic stress disorder
RCADS	Revised Child Anxiety and Depression Scale
rCBF	regional cerebral blood flow
RCT	randomised control trial
RDoC	Research Domain Criteria
ROI	region of interest
RSA	respiratory sinus arrhythmia
SAS	Social Aptitudes Scale
SCARED	Screen for Child Anxiety Related Emotional Disorders
SCAS	Spence Children's Anxiety Scale
SCICA	Semi-structured Clinical Interview for Children and Adolescents
SCQ	Social Communication Questionnaire
SDQ	Strengths and Difficulties Questionnaire
SFG	superior frontal gyrus
sgACC	subgenual anterior cingulate cortex
SMD	severe mood dysregulation
SMP	severe mood problems
SNAP	Special Needs and Autism Project
SPM	Statistical Parametric Mapping
SSRI	selective serotonin reuptake inhibitor
SVM	support vector machine
TD	typically developing
TEDS	Twins Early Development Study
ToM	theory of mind
TSST	Trier Social Stress Test
vlPFC	ventrolateral prefrontal cortex
vmPFC	ventromedial prefrontal cortex
WISC	Wechsler Intelligence Scale for Children
WASI	Wechsler Abbreviated Scale of Intelligence

Preface

Children and young people with ASD often experience high levels of co-occurring emotional problems that are devastating and can impact their lives and those of their families. Below is a telling personal account of a mother of a child with ASD (Park & Park, 2006):

Stressors in autism are highly idiosyncratic. For most autistic people, such questions ['What are you making?', 'What are you doing?'; my insertion] *would be no more stressful than for you and me. For Jessy, however, they elicit the most intense emotions she ever feels, and her overreactions may be extreme. The usual one, a loud, sudden, hostile, "Why do you ask me that?!" is frightening enough for the innocent questioner, who was only trying to start a conversation. If things go badly, as they may if there have been other stressors that day, the scene becomes terrifying, as Jessy cries and shrieks outs bizarre and piercing verbalizations that are audible through several closed doors. "Wee-alo, wee-alo" she screams, "La la," her face distorted, her heart pounding, every muscle in her body tense, her mouth open so wide we see her flattened tongue. (...) It is these overreactions to stress, rather than her remaining cognitive and communicative deficits, that are now the greatest difficulty for her and for those who live with her and work with her. Why do they elicit such intense emotion? We don't know.*

This account not only provides a vivid description of the problem and highlights the challenges that parents of a child with ASD may face, but also points toward the complex nature of emotional problems in young people with ASD. While the mother writes about 'stressors' and 'stressful reactions' that we often equate with anxiety, she also notes 'hostile' behaviours that are a common presentation of irritability. For researchers as well as clinicians, this immediately leads to questions about measurement: how do we make sure we capture these co-occurring symptoms accurately, study the mechanisms underlying them, and develop targeted treatments? These will be the main questions guiding the next two introductory chapters in this thesis.

Chapter 1 – Introduction to ASD and comorbid disorders

This chapter introduces autism spectrum disorder and provides a brief overview of the state of the evidence regarding prevalence and aetiology of the condition. Later in the chapter, I review the literature on psychiatric comorbidities in ASD, focusing on anxiety and irritability as the most common comorbid symptoms. I finish with a discussion of methodological limitations pertaining to the measurement of comorbid symptoms in youth with ASD, and introduce novel methods, such as pattern recognition techniques, that may address some of these challenges.

1.1 Introduction to ASD

1.1.1 Clinical presentation and diagnostic criteria

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterised by deficits in social interaction and communication, and by restricted, repetitive behaviours (APA, 2013). 'Infantile autism' was originally described by Leo Kanner (Kanner, 1943) based on observations of 11 children who presented with an *"inability to relate themselves in the ordinary way to people and situations"* and *"an anxiously obsessive desire for the preservation of sameness"*. In 1944, Hans Asperger described a group of children observed in his clinic who presented with similar features (Asperger, 1944).

ASD was first classified as a separate diagnostic category in the DSM-III (APA, 1980) as 'infantile autism' and described as a severe condition with significant delay in cognitive skills and language development. Subsequent diagnostic manuals recognised less severe manifestations of autism, with ICD-10 (WHO, 1992) and DSM-IV (APA, 2000) publishing diagnostic criteria for several separate ASD conditions, including autistic disorder, Asperger's syndrome, and pervasive developmental disorder-not otherwise specified (PDD-NOS). A diagnosis of 'autistic disorder' required the presence of impairments in the three domains of ASD--social interaction difficulties, difficulties in the use of language for communication, and restricted and repetitive behaviours and interests--before the age of 3 years. A diagnosis of Asperger's syndrome was

given if impairments in social interaction and repetitive behaviours were present in the absence of language delay and cognitive development deficits, whereas PDD-NOS was given to those who displayed significant impairments in one or more of the three ASD domains but did not meet criteria for autistic disorder or Asperger's syndrome due to subthreshold symptomatology and/or late age of onset.

In 2013, with the advent of DSM-5 (APA, 2013), the above diagnostic sub-categories were collapsed under the continuum of ASD (see Table 1.1 on page 26 for full diagnostic criteria for Autism Spectrum Disorder according to the DSM-5). The DSM-5 diagnosis of ASD requires that social communication and interaction deficits are accompanied by restricted and repetitive behaviours and interests, a change from DSM-IV criteria where a person with pervasive deficits in social reciprocity alone could be given a diagnosis of PDD-NOS. The changes in the diagnostic criteria for ASD as introduced into the DSM-5 have been widely debated. On one hand, the previous DSM-IV criteria for ASD have been criticised for lack of clarity leading to high rates of false positives (Gibbs, Aldridge, Chandler, Witzlsperger, & Smith, 2012; Wing, Gould, & Gillberg, 2011) and the proposed DSM-5 criteria were shown to significantly improve diagnostic specificity (McPartland, Reichow, & Volkmar, 2012). On the other hand, several studies suggested that introducing more stringent criteria into DSM-5 would inadvertently lead to the exclusion of some individuals who no longer meet criteria for ASD (Mandy, Charman, Gilmour, & Skuse, 2011; Mattila et al., 2011; McPartland, Reichow, et al., 2012), raising concerns about service eligibility and comparability between studies conducted historically vs. according to the new criteria, including research on comorbidity rates in youth with ASD (Rieske et al., 2015). These concerns might be partly remediated by the introduction of a new diagnosis – social (pragmatic) communication disorder – in the DSM-5, to characterise individuals with social communication deficits who do not meet criteria for an ASD diagnosis. Moreover, the addition of severity levels (see Table 1.1) may help clinicians identify the level of intervention needed based on the associated functional impairment.

1.1.2 Prevalence

Prior to the 1990s, the reported prevalence of autism was 4-5 cases per 10 000 (Brask, 1970; Lotter, 1966) although some studies reported higher prevalence of ASD-like impairments in social reciprocity (e.g., 21 per 10 000 reported by Wing & Gould, 1979). More recent epidemiological studies estimate the prevalence of ASD in children to lie between 0.6% and 1% (Baird et al., 2006; Fombonne, 2003, 2009), with a recent systematic review of epidemiological surveys reporting a median worldwide prevalence of 62 cases per 10 000 (Elsabbagh et al., 2012). The rise in ASD prevalence over time is believed to reflect the broadening of diagnostic criteria and increased awareness of the condition (Matson & Kozlowski, 2011; Wing & Potter, 2002).

The prevalence of ASD is consistently reported to be higher in males than in females, with a male:female ratio of approximately 3:1 (Baird et al., 2006) although higher proportions have also been reported (Elsabbagh et al., 2012). The diagnostic stability of ASD is high with estimates of 67-85% between the ages of 2 and 9 years (Charman et al., 2005; Lord et al., 2006). The lower estimates can to some extent be explained by movement within the different DSM-IV PDD categories (Lord et al., 2006), although the severity of ASD symptoms can also decrease with age (Anderson, Liang, & Lord, 2014; Piven, Harper, Palmer, & Arndt, 1996).

1.1.3 Aetiology

ASD is a highly heritable disorder, with heritability estimates of up to 93% (Bailey et al., 1995; Lichtenstein, Carlström, Råstam, Gillberg, & Anckarsäter, 2010). A recent meta-analysis of seven twin studies reported pooled heritability estimates between 64% and 91%, despite diagnostic heterogeneity (Tick, Bolton, Happé, Rutter, & Rijsdijk, 2016). Strong genetic influences are also suggested by concordance rates for ASD in monozygotic twins (36-92%) that are much higher than those reported for dizygotic twins (0-31%) (Bailey et al., 1995; Deng et al., 2015; Folstein & Rutter, 1977; Rosenberg et al., 2009). These varying estimates may reflect the heterogeneous nature of the disorder (Ronald, Happé, & Plomin, 2005; Viding & Blakemore, 2006) and suggest that ASD is likely to be underpinned by multiple genes, as well as environmental factors. In fact, only around 10-20% of cases with ASD are at present identifiable with known genetic causes (Geschwind, 2011). Evidence comes mainly from studies where ASD co-occurs with clearly-defined, rare single gene syndromes such as tuberous sclerosis (Folstein & Rosen-Sheidley, 2001), although common gene variants also contribute to ASD liability (Gaugler et al., 2014). Recently, two studies suggested the importance of shared environmental factors in ASD (Frazier et al., 2014; Hallmayer et al., 2011), although it is possible that these high estimates may be a statistical artefact caused by oversampling of concordant dizygotic twin pairs (Tick et al., 2016).

Second, several structural brain abnormalities have been associated with ASD, including reduced white matter volume (Ecker et al., 2012; Ke et al., 2008) and subtle neuroanatomic changes in the frontal, limbic, and cerebellar regions (Amaral, Schumann, & Nordahl, 2008; Courchesne et al., 2001; Sparks et al., 2002). However, these findings are not always replicated (Haznedar et al., 2000; Piven, Saliba, Bailey, & Arndt, 1997) and it has been suggested that multivariate pattern recognition techniques may be more fitting than univariate methods to study brain abnormalities in heterogeneous phenotypes such as ASD (Ecker & Murphy, 2014). In Section 1.2.3, I describe the pattern recognition methodology in more detail and suggest that the technique may also be promising for assessing comorbidities within ASD, possibly bypassing the limitations of diagnostic interviews when assessing psychopathology in individuals with severe communication difficulties.

Several cognitive processing abnormalities have also been implicated in the aetiology of ASD. Baron-Cohen, Leslie and Frith (1985) suggested that children with ASD may lack theory of mind (ToM), an ability to introspect their own thoughts and attribute mental states to others. Impaired ToM, also called mentalising, has been proposed to account for social communication impairments seen in ASD (Joseph & Tager-Flusberg, 2004). In addition, the neural correlates of ToM differ between individuals with ASD and typically developing controls, whereby adults (Castelli, Frith, Happe, & Frith, 2002) and children with ASD (Kana et al., 2015) show reduced functional connectivity between frontal and posterior brain regions when performing mentalising tasks. Prefrontal cortex dysfunction has also been proposed to account for more broad executive function deficits seen in ASD (Hill, 2004). Lastly, the weak central coherence account proposes that people with ASD have a detail-focused processing style, while typically developing individuals process stimuli within a wider context (Frith, 1989; Happé, 1999). This theory accounts for the remarkable 'savant' skills of some people with ASD (e.g., photographic memory) and could explain why children with ASD may sometimes be more interested in specific features of a toy rather than the toy itself. While weak central coherence does not account for all impairments frequently observed in ASD, Happé (1997) suggested that it may result in impairment when accompanied by other deficits, e.g. impaired ToM.

1.1.4 Categorical vs. dimensional approaches to ASD

Alongside changes in the diagnostic criteria for ASD, there has been a shift in how ASD is studied in research. Long before the advent of newer editions of the diagnostic manuals, it has been suggested that diagnosable autism may represent the most severe end of the spectrum of social communication difficulties within the general population, with Asperger's syndrome lying further along the continuum and towards typical development (Frith, 1991; Wing, 1981). With the increased recognition that autism-like impairments are also present below the clinical threshold for a diagnosis, especially in family members of affected individuals (Bolton et al., 1994; Constantino et al., 2006), the scope of ASD research has expanded into investigating ASD traits within the general population, also referred to as the "broader autism phenotype" (Piven, Palmer, Jacobi, Childress, & Arndt, 1997).

Evidence for the dimensional view of ASD comes from behavioural, neuroimaging and genetic studies (for a review, see Sucksmith, Roth, & Hoekstra, 2011). At the behavioural level, the distribution of ASD trait scores across a sample of children aged 7 to 15, randomly generated from an epidemiological dataset, is smooth; without obvious peaks that would suggest the presence of categorically distinct phenotypes (Constantino & Todd, 2003; Happé, Ronald, & Plomin, 2006). Furthermore, structural brain imaging studies in samples that include typically developing participants as well as those with a diagnosis of ASD report relationships between

increased ASD traits severity and decreased cortical gyrification in children (Blanken et al., 2015) as well as reduced white matter coherence in young adults (Gibbard et al., 2013). The differences persist after the most severely impaired participants are removed from the analyses and are not explained by differences in IQ, suggesting a dimensional relationship between structural brain abnormalities and ASD traits. An association between reduced cortical thickness and increased severity of ASD traits has also been reported in adults (Gebauer, Foster, Vuust, & Hyde, 2015), suggesting that these structural differences are stable across time. Moreover, ASD traits, similar to ASD diagnoses, also show stability across childhood in the general population (E. B. Robinson, Munir, et al., 2011) and are highly heritable (Tick et al., 2016), suggesting that the temporal stability may be underpinned by genetic influences (Holmboe et al., 2014; Piven, Palmer, et al., 1997; St Pourcain et al., 2013).

Research into the broad autism phenotype has been accompanied by the development of questionnaires to measure ASD traits in the general population, e.g. the Social Responsiveness Scale (Constantino, 2002), the Autism-Spectrum Quotient (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001), the Social Aptitudes Scale (Liddle, Batty, & Goodman, 2008), or the Social Communication Disorders Checklist (Skuse, Mandy, & Scourfield, 2005). Rutter and colleagues measured ASD traits with a semi-structured Family History Interview (Bolton et al., 1994; Rutter & Folstein, 1995) and identified 7.5% of their sample (which comprised 2360 relatives of patients with ASD) as belonging to the broader autism phenotype (Pickles et al., 2000). A comparable 6.0% of children from a population-based sample (Avon Longitudinal Study of Parents and Children, ALSPAC) scored within the range predictive of ASD based on the Social Communication Disorders Checklist (Kothari, Skuse, Wakefield, & Micali, 2013). Furthermore, 5.8% of children from a mainstream school sample scored above the cut-off on the Childhood Asperger Syndrome Test, and this cut-off identified children with ASD traits with the highest degree of specificity and sensitivity (Williams et al., 2005). Consistent with these prevalence estimates, and in line with the dimensional distribution of ASD traits, some researchers have used a pre-defined cut-off to identify participants scoring at the highest quantitative extremes on ASD measures. For example, the highest-scoring 5% in a population-derived twin sample, the Twins Early Development Study (TEDS), were classified as belonging to the broader autism phenotype in several studies (Colvert et al., 2015; Ronald, Happé, Price, Baron-Cohen, & Plomin, 2006). Also using the TEDS dataset, Robinson et al (2011) compared participants scoring in the highest 1%, 2.5%, and 5% of the sample on the Childhood Autism Spectrum Test and found no differences in heritability rates between the different extreme groups or between the extreme groups and the general population. This once again suggests that shared aetiological factors may be at play across the spectrum of ASD traits severity.

Table 1.1. Current diagnostic criteria for Autism Spectrum Disorder, as published in the DSM-5 (APA, 2013).

Autism Spectrum Disorder 299.00 (F84.0)
Diagnostic Criteria
[Please note: Due to copyright restrictions, the list of DSM-5 diagnostic criteria for Autism Spectrum Disorder has been removed from the electronic version of this thesis. The criteria have been reprinted with permission from the American Psychiatric Association in the print version of this thesis, held in the university library.]

1.2 Comorbid psychiatric conditions in youth with ASD: Prevalence and phenomenology

Apart from the core impairments in social interaction and communication, restricted interests and repetitive behaviours, young people with ASD often display high levels of psychiatric comorbidity. Even early on in development, toddlers and children with ASD show higher rates of comorbid disorders than typically developing (TD) children (for a review, see Mannion & Leader, 2013). These additional psychiatric problems persist from childhood to adolescence (Simonoff, Jones, et al., 2013) and remain functionally impairing in adulthood (Joshi et al., 2013). The rates of emotional and behavioural problems are higher in youth with ASD than in those with intellectual disability (Brereton, Tonge, & Einfeld, 2006). In a population-based study of children with ASD, 70% of children met criteria for at least one additional psychiatric disorder (3 months' prevalence), while 41% had two or more additional disorders (Simonoff et al., 2008). Anxiety disorders (42%), attention-deficit/hyperactivity disorder (ADHD; 28%), and oppositional defiant disorder (ODD; 28%) were the most frequent co-occurring psychiatric conditions in the sample. Comorbid psychopathology causes significant functional impairment for young people with ASD (Kaat, Gadow, & Lecavalier, 2013; Mattila et al., 2010; Simonoff, Jones, et al., 2013), elevates the risk of hospitalisation (Mandell, 2007), and impacts on the quality of life of their families (J. A. Kim, Szatmari, Bryson, Streiner, & Wilson, 2000; Lecavalier, Leone, & Wiltz, 2006). Mattila and colleagues reported an exponential decrease in the Children's Global Assessment Scale (CGAS) scores with every additional comorbid disorder among 9 to 16 year-old youth with ASD (Mattila et al., 2010). The discussion in this chapter will focus on the prevalence and phenomenology of the two most common comorbidities in ASD youth: anxiety (Simonoff et al., 2008) and irritability (McCracken et al., 2002; Robb, 2010).

1.2.1 Anxiety

1.2.1.1 Anxiety disorders in TD youth

Anxiety disorders are common in young people, with a cumulative incidence by the age of 19 ranging from 8% to 27% in the general population (Costello, Egger, & Angold, 2005). While anxiety can be an adaptive response that enables avoidance of danger, persisting or excessive degrees of anxiety and avoidance can become maladaptive and cause substantial functional impairment (Rosen & Schulkin, 1998). Current diagnostic manuals separate anxiety disorders into several discrete categories based on their primary features, e.g. generalised anxiety disorder (GAD), social anxiety disorder (or "social phobia"), separation anxiety, panic disorder, or post-traumatic stress disorder (PTSD). Obsessive-compulsive disorder (OCD) also features as an anxiety disorder in the DSM-IV and ICD-10 (but not in DSM-5) and describes a condition where intrusive, obsessive thoughts generate impairing levels of anxiety that result in compulsive behaviour. Cognitive behaviour therapy (CBT) is the most widely used treatment for anxiety in children and adolescents, with an effectiveness estimate of OR=7.85 compared with waitlist control, according to a recent meta-analysis of 26 randomised control trials (RCTs) (James, James, Cowdrey, Soler, & Choke, 2015), although authors note that evidence for the effectiveness of CBT compared to other active treatments is limited. Medication treatment, specifically with selective serotonin reuptake inhibitors (SSRIs), is comparably effective in reducing core symptoms of anxiety in children and young people (Ipser, Stein, Hawkridge, & Hoppe, 2009), and combined SSRI and CBT treatment is more effective than each single treatment alone (Walkup et al., 2008). Evidence suggests that childhood anxiety disorders (Last, Perrin, Hersen, & Kazdin, 1996) as well as the temperamental style of behavioural inhibition (Chronis-Tuscano et al., 2009) increase the risk of suffering from anxiety disorders later in life.

1.2.1.2 Anxiety phenomenology in ASD

Already in his original description of children with autism, Kanner (1943) observed that "*the child's behaviour is governed by an anxiously obsessive desire for the maintenance of sameness*", suggesting that anxiety is intertwined in the presentation of autism. Anxiety often features in accounts given by individuals with ASD themselves, e.g., "*It is the confusion that results from not being able to understand the world around me which, I think, causes all the fear. This fear then brings a need to withdraw*" (Joliffe, Landsdown, & Robinson, 1992). Anxiety has thus been conceptualised as both the consequence of ASD-specific impairments, as well as a factor driving ASD-related behaviours. For example, Despert (1965) suggested that stereotyped behaviours may act as an anxiety reducing strategy in children with autism; indeed higher levels of anxiety are associated with an increased frequency of repetitive behaviours in youth with ASD (Rodgers,

Glod, Connolly, & McConachie, 2012; Sukhodolsky et al., 2008) and parents of children with ASD report a more frequent use of repetitive behaviours as an emotion regulation strategy by their children, compared to parents of TD youth (Samson, Wells, Phillips, Hardan, & Gross, 2015). Nevertheless, stereotyped behaviours may also have a negative emotional valence for young people with ASD (Spiker, Lin, Van Dyke, & Wood, 2012), and different types of stereotyped behaviours have been found to relate differentially to anxiety (Spiker et al., 2012; Stratis & Lecavalier, 2013), underscoring the heterogeneous nature of anxiety manifestations and the associated coping strategies in this population.

Although distinguishing symptoms of anxiety from manifestations of ASD-specific impairments can be difficult (see Section 2.1.1 on artefactual comorbidity for a more detailed discussion), several studies have investigated the phenomenology of anxiety in ASD and compared it to that in TD youth. Unusual phobias that rarely present in TD children have been widely reported in ASD, including fears of mechanical objects, vacuum cleaners and beards, or the fear of loud noises and wind (Kanner, 1943; Mayes et al., 2013; Muris, Steerneman, Merckelbach, Holdrinet, & Meesters, 1998). The latter may be related to sensory over-sensitivity that is frequently observed in ASD. Gillott and colleagues took a broader perspective on anxiety symptoms and compared responses on the Spence Children's Anxiety Scale (SCAS) (Spence, Barrett, & Turner, 2003) by children with high-functioning ASD and TD controls aged 8 to 12 (Gillott, Furniss, & Walter, 2001). The authors reported a different pattern of responses between the groups, whereby children with ASD scored highest on the separation anxiety and OCD subscales, whereas TD children reported GAD, OCD, and social anxiety as the most prevalent symptoms. Using the parent-reported SCAS, Russell and Sofronoff (2005) found that children with Asperger Syndrome scored significantly higher on the overall measure of anxiety than clinically-anxious TD children. The pattern of anxiety symptoms between groups also differed in that children with Asperger Syndrome scored higher on the OCD and personal injury subscales. While these studies suggest that the presentation of anxiety disorders in ASD may be different to TD youth, important limitations need to be noted. The measure used has been developed for use with TD children, and although preliminary evidence suggests that the SCAS may be a useful screening tool for anxiety in youth with ASD (Zainal et al., 2014), without population norms the results should be treated with caution. In addition, as noted in Section 1.2.3, combining self and parent reports of anxiety in ASD may be advantageous in order to get a fuller picture of the child's psychopathology. One study adopted this approach with regards to OCD, additionally using the gold-standard, parent- and child-informed Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS) (Freeman, Flessner, & Garcia, 2011) semi-structured interview (Ruta, Mugno, D'Arrigo, Vitiello, & Mazzone, 2010). The disentanglement of OCD and ASD symptoms is a particular challenge. On one hand, stereotyped and rigid behaviours, common in ASD, may present similarly to compulsions as seen in OCD. On the other, it may be difficult to assess (due to self-reporting difficulties) whether such behaviours occur in response to an obsessive thought,

a criterion required for a diagnosis of OCD. Ruta and colleagues compared OCD symptoms between children with ASD and age, gender, and IQ-matched TD controls with and without OCD. While children with ASD and those with OCD both showed higher symptom severity compared to healthy controls, the quality of symptoms differed between those with ASD vs. OCD. The otherwise typically-developing OCD group reported more checking compulsions and obsessions regarding contamination and aggression, whereas children with ASD reported more hoarding and ordering behaviours. The ASD group showed an overall mild clinical impairment due to OCD, with a total CY-BOCS score of 15 compared to 22 in the OCD group (Ruta et al., 2010). This study is limited by the absence of a comorbid ASD+OCD comparison group. Since not all participants in the ASD group met clinical criteria for OCD, it is possible that the differences in symptom patterns are partly due to OCD symptom severity. In addition, the validity of using the CY-BOCS, a measure developed for use with TD children, in the ASD population has been questioned. Due to the particular difficulty with assessing obsessions, Scahill and colleagues proposed that only compulsion items be used with children with OCD, as symptoms that can be reported on reliably by the parents (Hallett, Lecavalier, et al., 2013; Scahill et al., 2014). On the other hand, a recent study comparing OCD treatment response among young people with ASD vs. TD controls found that CBT successfully decreased the severity of OCD symptoms in both groups (albeit to a lesser degree in the ASD group, possibly reflecting the need of more ASD-specific CBT for OCD modifications) (Murray, Jassi, Mataix-Cols, Barrow, & Krebs, 2015). Although treatment response alone does not constitute evidence for equivalent psychopathology, it provides an important insight into the possible similarities regarding OCD in ASD and TD youth. This is also true for other anxiety disorders, where adapted CBT has proven effective in reducing anxiety symptomatology within the ASD population (for recent meta-analyses of CBT treatment studies in youth with ASD, see Sukhodolsky, Bloch, Panza, & Reichow, 2013; Ung, Selles, Small, & Storch, 2015).

Finally, a carefully-designed study by Kerns et al (2014) assessed the phenomenology of anxiety in 59 young people with ASD, aged 7-17, using a variety of measures and reporting sources. Each participant underwent an extensive diagnostic assessment, with anxiety measured by one self-reported and two parent-reported scales, as well as the child and parent versions of the diagnostic semi-structured interview, Anxiety Disorders Interview Schedule (ADIS) (W. K. Silverman & Albano, 1996). Crucially, a supplement to the ADIS was developed in order to additionally investigate 'atypical' (non-DSM-IV-defined) anxiety, i.e. clinically-impairing symptoms of anxiety that are likely to represent an exacerbation of features related to ASD. 'Atypical anxiety' counterparts were added for each DSM-IV anxiety disorder under investigation. For instance, a rating of atypical anxiety rather than social phobia was given to children who showed consistent social fearfulness and discomfort in the absence of fear of negative evaluation. Kerns et al (2014) reported that 17% of the sample met criteria for DSM-IV defined anxiety, 15% only displayed atypical anxiety, while 31% showed both types. The most

common atypical anxieties that accounted for 22% of all clinically-significant symptoms were anticipatory anxiety around changes in routine, novelty and excessive worries about losing access to restricted interests. Interestingly, while traditional, DSM-IV-defined anxiety disorders were predicted by anxious cognitions and language ability, atypical symptoms were predicted by the convergence of traditional anxiety and ASD symptoms. This suggests that youth with ASD may experience both typical and atypical anxiety symptoms, the latter being exacerbated by their ASD-specific impairments.

1.2.1.3 Prevalence of anxiety disorders in youth with ASD

Although initial prevalence estimates varied widely (e.g., 11-84% as reviewed by S. W. White, Oswald, Ollendick, & Scahill, 2009), it is now accepted that anxiety disorders are one of the most common comorbid conditions in youth with ASD (Gjevik, Eldevik, Fjæran-Granum, & Sponheim, 2010; Leyfer et al., 2006; Simonoff et al., 2008). A recent meta-analysis reported that 39.6% of young people with ASD had at least one DSM-IV-defined anxiety disorder (Van Steensel, Bogels, & Perrin, 2011). The differences in prevalence rates reported across studies may be due to methodological factors, sample heterogeneity and recruitment strategies (see Table 1.2). As explained in detail in Section 2.1.1, comorbidity rates are likely to be overestimated in clinic-based samples due to referral or selection biases; the gold standard for establishing comorbidity rates would be based on a thorough diagnostic assessment in a large, epidemiological sample. To date, only one study employed this approach. Simonoff et al (2008) used the Special Needs and Autism Project (SNAP) sample of 112 children with ASD derived from an initial population cohort of 56 946 youth. Children (aged 10 to 14) were selected if they scored above 15 on the Social Communication Questionnaire (SCQ) (Rutter, Bailey, Lord, & Berument, 2003) and were diagnosed with ASD based on the gold standard parent-informed Autism Diagnostic Interview-Revised (ADI-R) (Lord, Rutter, & Le Couteur, 1994) together with the observational tool, Autism Diagnostic Observation Schedule-Generic (ADOS-G) (Lord et al., 2000). All children then underwent an in-depth diagnostic assessment for comorbid psychopathology (3 months' prevalence) using the parent-reported Child and Adolescent Psychiatric Assessment (CAPA) (Angold & Costello, 2000) . Simonoff et al (2008) found that 70% of their sample met DSM-IV-defined criteria for at least one additional disorder, with social anxiety being the most common diagnosis (29.2%). 41.9% of the children met criteria for at least one anxiety disorder. While the Simonoff et al (2008) study has the unique advantage of using a population-derived sample, other studies have employed a diagnostic interview schedule specifically designed to assess comorbidity in youth with ASD. The Autism Comorbidity Interview-Present and Lifetime (ACI-PL) (Lainhart, Leyfer, & Folstein, 2003) was designed with the view of limiting artefactual comorbidity due to overlapping diagnostic criteria or common phenomenological features between ASD and other disorders, such as anxiety. For example, a diagnosis of separation anxiety

according to the ACI requires that the anxiety is generated directly by separation from the caregiver and does not occur merely as a result of change in routine. The ACI is a modified version of the widely-used and validated (Ambrosini, 2000) Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS) (Kaufman et al., 1997) and was used by three studies to date to assess the prevalence of anxiety in children with ASD (Leyfer et al., 2006; Mazefsky, Kao, & Oswald, 2011; Mazefsky, Oswald, et al., 2012). Leyfer et al (2006) used a community sample of 109 children aged 5-17 and ascertained the diagnosis of ASD with the ADOS and ADI-R. Similarly to Simonoff et al (2008), 72% of the sample met diagnostic criteria for at least one DSM-IV-defined Axis 1 disorder. However, Leyfer et al (2006) reported a lower prevalence of social anxiety (13.2%) and relatively higher prevalences of OCD (37.2%) and specific phobia (44.3%) in their sample. While the lower estimate for social anxiety may be linked to the adoption of differential diagnosis considerations in the ACI, this does not explain the higher prevalence rates of OCD and phobias. These differences may be partly due to the age of participants. Children studied by Leyfer et al were relatively younger, and lower mean age was found to be associated with significantly higher prevalence rates of OCD in ASD in the recent meta-analysis (Van Steensel et al., 2011). An alternative explanation is that Leyfer et al investigated lifetime prevalence of comorbid conditions, while Simonoff et al concentrated on three months' prevalence. The ACI was also employed by Mazefsky et al (2012) in their study of 35 children with ASD from a clinic-based sample. Interestingly, the authors compared the rates of lifetime comorbid diagnoses as ascertained using the ACI with the number of prior diagnoses reported by the parents on a past psychiatric history questionnaire. The rate of agreement between the ACI and prior diagnoses was relatively high for anxiety, with 8/15 (53.3%) prior diagnoses of anxiety confirmed with the ACI (only surpassed by a 57.4% agreement for depression). However, none of the 4 prior diagnoses of OCD were confirmed, suggesting that the OCD symptoms in the sample might have been better accounted for by stereotyped behaviour and cognitive inflexibility inherent to ASD. Interestingly, anxiety disorders were also the most common previously undetected diagnoses in the sample, with five participants (14.3%) receiving a new diagnosis of anxiety based on the ACI. Table 1.2 summarises the reported prevalence rates of anxiety in other studies that have used parent-reported, semi-structured interviews. Most of these studies have used clinic-based samples which, as explained further in Section 2.1.1, may overestimate the true rates of comorbidity in the population of youth with ASD and high estimates reported in these studies should be treated with caution. Moreover, in some cases (Muris et al., 1998) the purpose of the study was explicitly stated as investigating anxiety in ASD in recruitment materials, which may explain a very high (84.1%) rate of anxiety reported in the sample.

Consistent with the dimensional conceptualisation of ASD, several studies have recently investigated associations between ASD traits and anxiety in typically developing young people. For example, Hallett and colleagues reported increased anxiety symptoms across all domains studied (GAD, OCD, panic, separation and social anxiety) in co-twins of children with ASD who

displayed a broad autism phenotype, compared to controls (Hallett, Ronald, et al., 2013). The dimensional approach has also been adopted with regards to comorbid 'traits', based on the view that research on subthreshold symptoms may be highly informative for the diagnosis and treatment of their more extreme manifestations (Plomin, Haworth, & Davis, 2009). Three studies have used the large, community-based TEDS sample for this purpose (Hallett, Ronald, Rijsdijk, & Happe, 2010, 2012; Scherff et al., 2014). In a sub-sample of 12-14-year-olds, Scherf et al (2014) measured internalising traits with the emotional problems subscale from the Strengths and Difficulties Questionnaire (SDQ) (R. Goodman, 2001), which comprises three questions about anxiety, one question about somatic symptoms, and one about low mood. Autistic traits were measured with the Autism Spectrum Quotient. Scherf et al (2014) reported significant, moderate correlations between autistic and internalising traits (r=0.30), across all ASD sub-domains, partially replicating the findings by Hallett et al (2012) who reported moderate phenotypic and genetic correlations between generalised anxiety and ASD-like communication impairments and repetitive behaviours in a younger sample of 7 and 8 year-olds. Importantly, the two studies used different measures of internalising and ASD traits, suggesting that the association between these traits in the general population is not measure-specific.

Table 1.2. Summary of studies that have examined the prevalence of comorbid anxiety[1] and/or irritability in youth with ASD using parent-reported, semi-structured interviews.

Citation	Sample characteristics				Comorbidity assessment (timeline)	Prevalence of anxiety disorders	Prevalence of irritability
	source	n	age in years	full scale IQ			
Amr et al (2012)	Clinic-based sample	60	8.4 ± 1.8	60.9 ± 20.9 (20-105)	SCICA (-)	Any anxiety (58.3%); OCD (55%), specific phobia (40%), GAD (10%), separation anxiety (8.3%).	CD (23.3%).
Bradley et al (2004)	Community sample	12	16.3 ± 2.2	< 40	DASH-II (-)	42% reached clinical threshold for anxiety.	Participants with ASD scored significantly higher than TD controls on the emotional lability subscale of the DASH-II, but did not differ on the aggression/conduct subscale.
Caamaño et al (2013)	Clinic-based sample & autism charity	25	12.8 ± 2.9	97.9 ± 27.6	K-SADS (-)	OCD (48%), social phobia (40%), agoraphobia and specific phobia (36%), GAD (32%), separation anxiety (28%), panic (20%).	ODD (48%), CD (16%). "Irritability and anger" symptoms from K-SADS Affective Disorders supplement: 20% met threshold, 28% had sub-threshold symptomatology.
De Bruin et al (2006)	Clinic-based sample	94	8.5 ± 1.9 (6-12)	91.2 ± 17.4 (55-120)	DISC-IV (1 year)	Any anxiety (55.3%); specific phobia (38.3%), social phobia (11.7%), separation anxiety (8.5%), agoraphobia (6.4%), OCD (6.4%), GAD (5.3%), panic disorder (1.1%).	ODD (37.2%), CD (9.6%).
Gjevik et al (2010)	Special school for students with ASD	71	11.8 ± 3.3 (6-18)	Nonverbal IQ: 65.2 ± 29.6 (30–129)	K-SADS (Current)	Any anxiety (42%), social phobia (31%), OCD (10%), specific phobia (7%), GAD (0%), separation anxiety (0%).	ODD (4%), CD (2.8%).

[1] OCD was recently classified into a separate category of "obsessive-compulsive and related disorders" in the DSM-5, a change reflecting its putative aetiological relatedness to a group of disorders including body dysmorphic disorder (BDD) and trichotillomania. The reclassification of OCD has been widely debated, largely due to limited evidence for aetiological overlap between OCD and the 'related disorders' (e.g. (Monzani, Rijsdijk, Harris, & Mataix-Cols, 2014), and phenotypic similarities between OCD and anxiety disorders, also with regards to treatment response (for a review, see Storch, Abramowitz, & Goodman, 2008). Since most studies included in the table used DSM-IV criteria, prevalence of OCD is also reported.

Green et al (2000)	Clinic-based sample	20	13.8 (11-19)	92.2 ± 17.7 (71-141)	IWS (3 months)	GAD (35%), OCD (25%), simple phobia (10%).	ODD (25%), CD (25%). 2 or more parent-reported specific symptoms of: irritability (55%), defiance to parents (35%), temper tantrums (25%).
Hepburn et al (2014)	Clinic-based sample enriched for anxiety	42	10.9 ± 1.8 (8–14)	98.4 ± 15.0 (63–129)	K-SADS (Current)	N/A	ODD (19%), CD (4.8%).
Joshi et al (2010)	Clinic-based sample	217	9.7 ± 3.6 (3-17)		K-SADS (Lifetime)	61% met criteria for 2 or more anxiety disorders. Specific phobia (37%), separation anxiety (37%), GAD (35%), agoraphobia (35%), social anxiety (28%), OCD (25%), panic disorder (6%), PTSD (2%).	ODD (73%), CD (22%).
Kerns et al (2014)	Clinic-based sample	59	10.5 ± 2.6 (7-17)	104.7± 191 (67-158)	ADIS-C/P (-)	Any anxiety (63%), including DSM-IV defined (17%), 'atypical' (15%), both (31%). Specific phobia (30%), GAD (22%), social phobia (17%), separation anxiety (10%), OCD (2%).	Not assessed
Leyfer et al (2006)	Community (schools, parent groups)	109	9.2 ± 2.7 (5-17)	82.6 ± 23.4 (42–141)	ACI-PL (Lifetime)	Specific phobia (44.3%), OCD (37.2%), separation anxiety (11.9%), social phobia (7.5%), GAD (2.4%, n=1/41)	ODD (7.0%, n=6/86).
Mandy et al (2014)	Clinic-based sample	216	9.1 ± 2.1 (3-16)	Verbal IQ: 93.6 ± 19.9 (40-153)	3Di (-)	Not assessed	Angry and irritable symptoms 'definitely present': angry and resentful (65%), temper loss (64%), touchiness (52%). SDQ 'definite problems' range: conduct (56.5%), emotional (49.1%).
Mattila et al (2010)	80% clinic-based, 20% community sample	50	12.7 (10-16)	> 75	K-SADS (Current)	Any anxiety (42%), specific phobia (28%), OCD (22%), social phobia (4%), agoraphobia (4%), separation anxiety (2%), panic (2%).	ODD (16%), CD (2%).
Mazefsky et al (2011)	Clinic-based sample	38	12 ± 2 (10-17)	105 ± 17 (71-144)	ACI-PL (Lifetime)	Any anxiety (31.6%); social anxiety (13.2%), specific phobia (13.2%), GAD (7.9%), separation anxiety (7.9%), OCD (2.6%), panic (0%).	Not reported

Mazefsky et al (2012)	Clinic-based sample	35[2]	12.1 ± 2 (10-17)	105 ± 17 (71-144)	ACI-PL (Lifetime)	Any anxiety (37.1%); OCD (2.9%).	ODD (11.4%). Only 2/10 prior diagnoses of ODD were upheld based on ACI-PL.
Mukaddes et al (2010)	Clinic-based sample (Asperger Syndrome)	30	11.0 (7.0 – 15.5)	106.5 (82 – 138)	K-SADS (Lifetime)	Any anxiety (73.3%), specific phobia (46.7%), OCD (36.7%), GAD (16.7%), social anxiety (13.3%), separation anxiety (10%), panic (0%).	ODD (30%), CD (0%)
Mukaddes et al (2010)	Clinic-based sample (Autistic Disorder)	30	10.3 (6.2 – 14.4)	90.5 (70 -127)	K-SADS (Lifetime)	Any anxiety (83.3%), specific phobia (60%), OCD (37.7%), separation anxiety (16.7%), social anxiety (13.3%), GAD (3.3%), panic (3.3%).	ODD (33.3%), CD (3.3%).
Muris et al (1998)	Clinic-based sample	44	9.7 ± 4.8	79.5 (59-116)	DISC 2.3 (6 months)	Any anxiety (84.1%); simple phobia (63.6%), agoraphobia (45.5%), separation anxiety (27.3%), overanxious disorder (22.7%), social phobia (20.5%), avoidant disorder (18.2%), OCD (11.4%), panic disorder (9.1%).	Not assessed
Reinvall et al (2016)	Clinic-based sample	60	11.6 ± 2.5	105.5 ± 14.5	DAWBA, diagnosis by psychiatrist based on responses	Specific phobia (20%), GAD (10%), OCD (8.3%), social phobia (1.7%), PTSD (1.7%), separation anxiety (1.7%), panic/agoraphobia (0%).	ODD (8.3%), CD (1.7%).
Salazar et al (2015)	Clinic-based sample	101	6.7 ± 1.1 (4-9)	66.4 ± 28.0 (19-120)	PAPA (3 months)	Any anxiety (78.9 %); GAD (66.5%), specific phobia (52.7%), separation anxiety (18.6%), agoraphobia (18%), social phobia (15.1%), panic disorder (3.1%).	ODD (28.7%), CD (2.0%).
Simonoff et al (2008)	Population-derived sample	112	11.5 (10-14)	72.7 ± 21.6 (19-124)	CAPA (3 months)	Any anxiety (41.9%); social anxiety disorder (29.2%), GAD (13.4%), panic disorder (10.1%), simple phobia (8.5%), OCD (8.2%), agoraphobia (7.9%), separation anxiety (0.5%).	ODD (28.1%), CD (3.2%).
van Steensel et al (2013)	Clinic-based sample	40	11.1 ± 2.8 (8-18)	n=2 below 70	KID-SCID (-)	Any anxiety (27.5%), specific phobia (12.5%), social anxiety (10%), OCD (7.5%), GAD (5%), separation anxiety (2.5%).	ODD (22.5%), CD (2.5%).

[2] These participants were also included in the Mazefsky et al (2011) sample; however the Mazefsky et al (2012) study additionally reported the prevalence of ODD.

Witwer et al (2010)	Clinic-based sample	61	11.2 ± 3.8 (6-17)	68.4 ± 23.3 (42-150)	ChIPS (1 month)	Any anxiety (41.9%); social anxiety (29.2%), GAD (13.4%), panic (10.1%), specific phobia (8.5%), OCD (8.2), agoraphobia (7.9%), separation anxiety (0.5%).	ODD (75.4%), CD (49.2%). 100% reported some ODD symptoms, 88.5% reported impairment due to ODD symptoms. 37.7% displayed ODD symptom "angry a lot of time".
Wozniak et al (1997)	Clinic-based sample	66	9.8 ± 3.8	n=9 with "mental retardation"	K-SADS (Lifetime)	Two or more anxiety disorders (37.9%); agoraphobia (30.3%), GAD (28.8%), separation anxiety (24.2%), specific phobia (19.7%), OCD (15.2%), social anxiety (13.6%), panic (6.1%).	ODD (59.1%), CD (21.2%).

3Di, Dimensional, Developmental and Diagnostic Interview. *ACI-PL*, Autism Comorbidity Interview-Present and Lifetime. *ADIS-C/P*. Anxiety Disorders Interview Schedule: Child and Parent Versions. *CAPA*, Child and Adolescent Psychiatric Assessment. *CD*, conduct disorder. *ChIPS*, Children's Interview for Psychiatric Symptoms. *DASH-II*, Diagnostic Assessment for the Severely Handicapped-II. *DAWBA*, Development and Well-Being Assessment. *DISC*, Diagnostic Interview Schedule for Children. *GAD*, generalised anxiety disorder. *IWS*, Isle of Wight Semistructured Informant Interview. *KID-SCID*, Structured Clinical Interview for DSM-IV Childhood Diagnoses. *K-SADS*, Kiddie Schedule for Affective Disorders and Schizophrenia. *OCD*, obsessive compulsive disorder. *ODD*, oppositional defiant disorder. *PTSD*, post-traumatic stress disorder. *TD*, typically developing. *SCICA*, Semi-structured Clinical Interview for Children and Adolescents.

1.2.2 Irritability

Irritability, defined as easy annoyance and proneness to anger, is a common and impairing symptom in children and adolescents (Brotman et al., 2006; Leibenluft, Cohen, Gorrindo, Brook, & Pine, 2006; Mikita & Stringaris, 2013; Vidal-Ribas, Leibenluft, Valdivieso, Brotman, & Stringaris, 2016) that is associated with long-term adverse outcomes (Leibenluft, 2011). However, the nosological status of irritability, unlike that of anxiety disorders described in the previous section, is still unclear. The lack of an established diagnostic category for irritability impacts on its reported prevalence rates, and may contribute to inconsistencies in existent research that reports on irritability phenomenology. Keeping these limitations in mind, in this section I am going to provide an overview of the research on irritability in young people, mentioning different conceptual approaches (categorical and dimensional) to studying irritability. When referring to irritability as 'comorbid' with ASD, I use this to describe the co-occurrence of ASD with irritability that has reached a certain threshold, not necessarily implying that irritability constitutes a separate diagnostic category.

1.2.2.1 Irritability in TD youth

Irritability has long been treated as one of the non-specific symptoms present in the criteria for multiple different psychiatric disorders (both externalising and internalising), not unlike concentration difficulties (APA, 2013). As such, although commonly observed in clinical samples as part of the presentation of children and adolescents, irritability has not been extensively studied on its own. In 2003, Leibenluft and colleagues introduced a category of "severe mood dysregulation" (SMD) that described chronic and severe irritability with frequent and developmentally inappropriate temper outbursts, along with negatively-valenced mood in between outbursts (Leibenluft, Charney, Towbin, Bhangoo, & Pine, 2003). SMD was introduced in response to the 'bipolar controversy' that has seen the rate of paediatric bipolar diagnosis in the US to rise sharply (Blader & Carlson, 2007) based on the premise that mania in children may present as chronic, non-episodic irritability (Wozniak et al., 1995) (for a review, see Mikita & Stringaris, 2013). While initially only existent in the research domain, the SMD category led to the introduction of a new, similarly-defined, diagnostic category in the DSM-5-- disruptive mood dysregulation disorder (DMDD) (APA, 2013). Some have questioned the value of introducing DMDD as a separate nosological entity (Axelson et al., 2012). However, the main advantage of introducing DMDD is that it provides a diagnostic home for young people who suffer from substantial irritability in the absence of another diagnosable disorder, and who could otherwise miss the opportunity for treatment (Mikita & Stringaris, 2013). Indeed, accurate identification and timely treatment of irritability may have

important implications for the child's prognosis, as childhood irritability has been shown to increase the risk of depression and anxiety later in life (Burke, 2012; Stringaris & Goodman, 2009a).

The lifetime prevalence of SMD has been reported as 3.3% in children aged 9 to 19 (Brotman et al., 2006), same as the 3.3% estimate of DMDD reported in preschoolers (Copeland, Angold, Costello, & Egger, 2013). However, the reliability and validity of DMDD has been questioned, with poor diagnostic agreement between clinicians reported in the DSM-5 field trial (Regier et al., 2013), as well as poor test-retest reliability and diagnostic stability of DMDD (Axelson et al., 2012; Regier et al., 2013) and SMD (Brotman et al., 2006). With the lack of an empirically-derived cut-off for what constitutes 'pathological' irritability, and the above-mentioned controversies surrounding the DMDD diagnostic category, most existent research on irritability comes from studies that have investigated it as a dimension. Several independent exploratory and confirmatory factor analyses have identified irritability as a distinct dimension within ODD, next to 'headstrong' and 'hurtful' dimensions (Aebi, Plattner, Metzke, Bessler, & Steinhausen, 2013; Burke et al., 2014; Herzhoff & Tackett, in press; Krieger et al., 2013; Stringaris, Zavos, Leibenluft, Maughan, & Eley, 2012). Example items that feature in the irritability dimension include "temper tantrums or hot temper" and "sudden changes in mood or feelings" from the Child Behavior Checklist (CBCL) questionnaire (Achenbach, 1991). Validating the status of irritability as an independent construct are also its distinct cross-sectional and longitudinal associations with other psychopathology. Unlike the headstrong and hurtful dimensions of oppositionality that are associated with antisocial behaviours, irritability in young people consistently predicts internalising symptoms (Stringaris, Cohen, Pine, & Leibenluft, 2009; Stringaris & Goodman, 2009a, 2009b) and was reported to display a significantly stronger phenotypic relationship and genetic correlation with depression than with delinquency (Stringaris, Zavos, et al., 2012). A meta-analysis of 12 studies of chronic irritability in youth (n=7594) found that chronic irritability significantly predicted depression, anxiety, and ODD, but not bipolar disorder, conduct disorder, or ADHD in the general population (Vidal-Ribas et al., 2016). Importantly, irritability in young people is associated with long-term adverse outcomes (Copeland, Shanahan, Egger, Angold, & Costello, 2014; Dougherty et al., 2013; Stringaris et al., 2009), even after controlling for baseline psychopathology (Vidal-Ribas et al., 2016). Finally, while research on treatments and interventions for irritability in youth has been scarce, evidence is emerging for the effectiveness of parent training in reducing irritability symptoms among children with ODD (Scott & O'Connor, 2012), and low-dose risperidone treatment was found effective in SMD youth (Krieger et al., 2011).

1.2.2.2 Irritability phenomenology and prevalence in ASD

ASD diagnosis was an exclusion criterion in studies with SMD, and the DSM-5 specifies that DMDD symptoms should not be secondary to another medical or neurological condition (APA, 2013). It is therefore not surprising that only a handful of studies investigated irritability symptoms in ASD directly. Simonoff et al (2012) developed a scale of "severe mood problems" (SMP) for the purposes of their study of 91 adolescents with ASD from the SNAP cohort assessed at age 16, in order to allow for comparisons with similarly-defined SMD. The SMP scale comprised four items from the parent-reported Profile of Neuropsychiatric Symptoms (PONS) (Santosh, Baird, Pityaratstian, Tavare, & Gringras, 2006): 'explosive rage', 'low mood', 'depressive thoughts' and 'labile mood'. Several findings reported by Simonoff et al (2012) suggest that SMP in ASD are a similar construct to SMD in TD youth. Cross-sectionally, SMP were associated with the conduct and emotional subscales of the SDQ, and the association with conduct problems was driven by the SDQ item 'often has temper tantrums or hot tempers', one of the defining symptoms of irritability. The association between SMP and affective symptoms held longitudinally, with parent- and teacher-rated emotional problems at 12 predicting SMP at age 16, consistent with previously-reported longitudinal associations between SMD and affective problems in TD youth (Brotman et al., 2006; Stringaris & Goodman, 2009a). SMP were also associated with maternal mental health problems reported at time 1, consistent with the increased risk of affective disorders in parents of children with SMD (Brotman et al., 2007). More recently, Mayes et al (2015) compared the frequency of DMDD symptoms in a large clinic-based sample of high-functioning children with ASD (n=580), ADHD (n=827), and healthy controls (n=186), aged 6 to 16. They used two items from the mother-reported Pediatric Behavior Scale (PBS) (Lindgren & Koeppl, 1987) that mirror the DSM-5 diagnostic criteria for DMDD: 'irritable, gets angry or annoyed easily' and 'loses temper, has temper tantrums' in the previous 2 months. Children with ASD scored the highest on DMDD symptoms, with 45% displaying irritability and temper tantrums that were significantly impairing 'often' or 'very often' compared to 39% in ADHD, and 3% in healthy controls (Mayes et al., 2015). This was true even after controlling for other ODD symptoms in the ASD sample, and 10% of children with ASD and DMDD symptoms did not meet criteria for ODD, suggesting clinical importance of recognising irritability as an independent, impairing symptom in ASD youth.

Similarly to irritability research in TD youth, most ASD studies to date have focused on ODD as an overarching diagnostic category. As shown in Table 1.2, prevalence estimates for ODD in ASD youth vary widely, from 4%-7% in community samples (Gjevik et al., 2010; Leyfer et al., 2006), through 28% in a population-based study (Simonoff et al., 2008), up to over 70% in clinically referred samples (Joshi et al., 2010; Witwer & Lecavalier, 2010). There

are several possible explanations for this broad range of prevalences. Apart from sample type (population-based vs. clinic) and timeline for reporting of relevant symptoms, some have argued that ODD symptoms may be an epiphenomenon of ASD. For example, using the ACI, Mazefsky et al (2012) found that only 2 out of 10 historic diagnoses of ODD were upheld after assessing ODD symptoms with the ASD-specific diagnostic interview. In contrast, evidence from a study by Gadow and colleagues suggests that ODD is a valid clinical phenotype in children with ASD (Gadow, DeVincent, & Drabick, 2008). The authors compared 608 children with ASD to 326 TD children from a clinic sample and 800 healthy controls. They found similar clinical features of ODD across samples with regard to co-occurring psychiatric symptoms, whereby ODD was clearly differentiated from ADHD as well as comorbid ODD+ADHD, and children with both ODD and ADHD were the most impaired group in terms of symptom severity, medication use, and environmental adversity. The results suggest that irrespective of ASD severity, symptoms of ODD can be differentiated from other externalising symptoms (ADHD) and that similar risk factors operate across the spectrum of ASD severity, supporting diagnostic validity of ODD in ASD. Furthermore, a study in 216 children with ASD reported comparable cross-sectional associations between the three dimensions of ODD as found in TD youth, whereby irritability predicted internalising but not externalising problems, whereas the headstrong and hurtful dimensions were associated with externalising but not internalising symptoms (Mandy et al., 2014). The separability of irritable symptoms from other dimensions of ODD strengthens the argument that irritability should continue to be investigated as a separate construct within ASD; indeed several studies have reported on irritability symptoms as well as the ODD diagnosis (see Table 1.2). In addition, the parallels between ODD dimensionality in ASD and TD youth suggest that conduct problems and severe, aggressive behaviours are likely to be separate from irritability symptoms that may or may not lead to aggression. Consistent with this view, the prevalences of conduct disorder and ODD differ markedly in ASD youth, whereby rates of conduct disorder are usually lower than those of ODD (see Table 1.2).

The recognition of ODD in ASD is also important clinically. Although in some cases ODD symptoms may be better explained by ASD-driven behaviours (e.g., anger in response to changes in routine) (Mazefsky, Oswald, et al., 2012), not documenting it as an additional and impairing problem could lead to the child not accessing treatments and interventions proven effective in reducing ODD symptoms in TD youth. Evidence is emerging for the effectiveness of antipsychotics in reducing the severity of irritability symptoms in youth with ASD (for a review, see Elbe & Lalani, 2012). A recent meta-analysis of 11 RCTs reported that risperidone and aripiprazole were the most effective in reducing irritability symptoms as measured by the irritability subscale of the Aberrant Behavior Checklist (ABC) (Aman, Singh, Stewart, & Field, 1985) (Fung et al., 2016). However, it was not possible to determine which

individual symptoms were mostly improved by medication, and the ABC irritability subscale includes items relating to severe behavioural difficulties and aggression, which do not always demonstrate in children with severe irritability (Leibenluft, 2011; Stringaris, Goodman, et al., 2012). In addition, aripiprazole and risperidone treatment was associated with significant levels of side-effects (Fung et al., 2016), prompting the discussion on other, non-pharmacological interventions for irritability in ASD (McGuire et al., 2016). Indeed, emerging evidence suggests that parent training (Bearss et al., 2015; Sellinger & Elder, 2016), and CBT for anger management (Scarpa & Reyes, 2011; Sofronoff, Attwood, Hinton, & Levin, 2006) may be effective in reducing externalising symptoms in youth with ASD.

1.2.3 Measurement issues

One challenge pertaining to the research on mood and anxiety problems in ASD is the measurement of its symptoms. The nature of ASD – social communication impairments that reduce the individual's ability to describe mental states – makes it difficult for clinicians to decide whether a young person with ASD is also suffering from comorbid psychiatric symptoms. On top of problems with communication, around half of young people with ASD also suffer from global learning difficulties (Elsabbagh et al., 2012; Grzadzinski, Huerta, & Lord, 2013) that further impair their ability to understand complex questions and describe their personal experiences. These factors limit the value of the psychiatric interview with the child, which is the gold standard for diagnosing mood and anxiety problems in TD youth. Instead, most studies examining comorbidities in ASD youth have relied on parent-informed diagnostic interviews. Observation and parent- or teacher-ratings, while helpful in assessing behaviours, are often suboptimal for the assessment of mood states because they require inference about another person's internal state (Angold, 2002). As a result, mood problems may not be accurately recognised and therefore not treated in young people with ASD. On the other hand, there is also a risk of over-diagnosis and inappropriate treatment, e.g. in the case of depression, because many young people with ASD show limited affect, which may be misinterpreted as low mood. These difficulties with assessment are reflected in the literature on the prevalence of depression in youth with ASD, which varies between 0.5% and 80% and significant amount of the variation is due to problems with measurement (Stewart, Barnard, Pearson, Hasan, & O'Brien, 2006; Walker & Stringaris, in preparation). Lastly, several screening measurement scales used to assess mood and anxiety in TD children have not been validated in the ASD population.

Recent studies began to address these problems by assessing the psychometric properties of existing measurement scales in ASD youth and examining child-parent

agreement on the reported symptoms. A meta-analysis of studies in youth with ASD and/or learning difficulties showed moderate cross-informant agreement, with parent-child correlations of .42 for internalising problems and .44 for externalising problems (Stratis & Lecavalier, 2015). While parents and children show significant agreement when reporting on symptoms, moderate agreement estimates suggest that a multi-informant approach is recommended to obtain a comprehensive picture of the child's functioning across different contexts. Consistent with this argument, Knott and colleagues reported that although parents of children with ASD reported lower levels of social functioning than did the children themselves, the children still recognised their social interaction difficulties and provided valid information on the negative outcomes of these difficulties (Knott, Dunlop, & Mackay, 2006). Furthermore, among all SCAS items, those asking about overt manifestations of anxiety showed the strongest child-parent agreement in ASD youth (Magiati, Chan, Tan, & Poon, 2014), suggesting that more covert manifestations of anxiety may be missed by the parents. Emerging psychometric evidence also suggests the usefulness of self-reported anxiety scales in children with high-functioning ASD, although findings have been mixed. Van Steensel et al (2013) reported a good internal consistency of the Screen for Child Anxiety Related Emotional Disorders (SCARED) (Birmaher et al., 1997) in high-functioning children with ASD; the SCARED also showed convergent validity with the ADIS interview schedule. The child-parent agreement was moderate at .41-.42, somewhat lower than the corresponding .52-.56 in the comparison sample of TD children with anxiety disorders (Van Steensel, Deutschman, & Bögels, 2013). Importantly however, the authors reported that while sensitivity of the SCARED was high in both ASD and TD groups, the diagnostic specificity for particular anxiety diagnoses was poorer in the ASD group (Van Steensel, Deutschman, et al., 2013), possibly reflecting a degree of 'atypicality' in anxiety manifestations, as described previously in Section 1.2.1.2. Consistent with this possibility, White and colleagues reported that while similar latent factors emerged from the self-reported Multidimensional Anxiety Scale for Children (MASC) (March, Parker, Sullivan, Stallings, & Conners, 1997) in high-functioning ASD and TD youth, item covariances and relations among factor scores were different (S. W. White et al., 2015).

With regards to irritability, no existing scales have so far been validated for use with children with ASD. In fact, even in TD youth, most instruments used in irritability research were created from existing scales such as the CBCL, and few were designed specifically to measure irritability (Vidal-Ribas et al., 2016). One exception is the Affective Reactivity Index (ARI) (Stringaris, Goodman, et al., 2012), and the reliability of this scale in ASD youth is examined in Chapter 3. Another issue with regards to irritability measurement in ASD is that past research has sometimes used the term to describe severe behavioural difficulties such as verbal or physical aggression (Aman et al., 1985). This is different to how the term 'irritability'

is used in TD youth, where it refers to a mood problem that may or may not lead to aggression (Leibenluft, 2011), limiting comparisons that can be drawn between TD and ASD populations.

While questionnaire measures may provide some insight into mood and anxiety problems of youth with ASD, more elaborate methods are needed to ensure that comorbid psychiatric symptoms are not missed, especially in those children with ASD who have significant problems with reporting on their emotions or have co-occurring learning difficulties. More subtle behavioural aspects of anxiety can be captured with computer-based facial expression tracking, a technique found to predict changes in cortisol in ASD youth during experimentally-induced stress over and above parent-reported trait anxiety (Kaurin et al., submitted). Moreover, recent advances in neuroimaging have made multivariate analysis techniques (also called 'pattern recognition') a potentially useful tool for investigating mood states in youth with ASD. Pattern recognition, whereby specific patterns of symptoms that distinguish between groups are identified based on brain activation, has been successfully applied to discriminate between children with ASD and TD controls (Jiao et al., 2010; Lim et al., 2013; Uddin et al., 2013), and between children with depression and healthy controls (Wu et al., 2015). A recent systematic review of pattern recognition in psychiatric diagnostics concluded that the technique may prove particularly useful in cases where clinical uncertainty is high (Wolfers, Buitelaar, Beckmann, Franke, & Marquand, 2015). This may be promising for diagnosing poorly identifiable conditions such as depression in ASD, which is arguably less 'outwardly' manifested than irritability or anxiety, and as such is difficult to report on by parents and other informants. The third study in this thesis (Chapter 5) tests the feasibility of pattern recognition for identifying mood states in TD youth, a first step towards developing such techniques for use with children with ASD.

1.3 Interim summary

Studies investigating the phenomenology of anxiety and irritability in youth with ASD have been limited by problems with measurement, with paucity of ASD-specific measures and lack of population-specific norms making it difficult to disentangle comorbid symptoms from ASD-specific impairments. This may have influenced the prevalence estimates for comorbid disorders in youth with ASD, and the development of measures such as the ACI, together with the use of population-based samples, has the potential of providing more precise estimates. Despite these shortcomings, the breadth of existing evidence suggests that children and young people with ASD suffer from high levels of impairing comorbidities, in particular anxiety and irritability. Understanding the aetiological mechanisms behind the co-occurrence of anxiety

and/or irritability in youth with ASD may help develop effective, targeted interventions for these problems, and will be the focus of discussion in the next chapter.

Chapter 2 - Possible mechanisms underlying mood and anxiety problems in ASD

In the previous chapter I reviewed studies that examined the prevalence of anxiety and irritability in young people with ASD. While comorbidity rates in youth with ASD are substantial, the reasons for this widely reported overlap remain poorly understood. In this chapter, I first describe theoretical models that have been put forward to explain the co-occurrence of two or more disorders in a single individual. I then move on to discussing reward processing and physiological responsiveness to stress as two possible processes that may underlie the overlap between ASD, irritability, and anxiety. Understanding the pathophysiological mechanisms underlying comorbidity has important clinical implications, in that treatments developed for a single condition may need to be adapted to remain effective in instances where one condition co-occurs with another.

2.1 Models of comorbidity

The term comorbidity was coined by Feinstein (1970) to describe the co-occurrence of two or more diagnoses in a single individual. While comorbidity may present a challenge to clinicians and researchers--since it is sometimes difficult to disentangle the individual symptoms that make up each co-occurring disorder (see Section 1.2.1.2 on phenomenology of anxiety in ASD) – it also presents an opportunity for researchers who are interested in studying the underlying causes of the overlap. In this section I describe reasons that may underlie observed comorbidity rates: artefactual, chance, and 'true' comorbidity models. Where appropriate, I relate these comorbidity models to ASD and emotional problems, as well as other disorders that illustrate specific models particularly well.

2.1.1 Artefactual comorbidity

First I am going to present possible reasons for artefactual comorbidity that are likely to provide biased estimates of comorbidity rates between disorders.

Referral and selection biases. Comorbidity rates in clinical samples are likely to be affected by several kinds of selection biases. Berkson bias (Berkson, 1946) refers to the statistically higher probability of comorbid cases to be part of a clinical sample. The probability of a comorbid case being referred is a function of the combined probability of each disorder being referred separately. Berkson bias occurs when two disorders both merit referral or admittance to hospital, but not all cases are referred/admitted. Second, it has been observed that those with multiple (Costello et al., 1996) and more severe symptoms (Angold et al., 1998) are more likely to be in treatment and therefore to form part of a clinical sample. There is also a possibility of a referral bias whereby a comorbid case is more likely to be referred to a specialised service or a clinician with a specific interest in a certain comorbidity pattern (Caron & Rutter, 1991), therefore inflating the true rate of that comorbidity estimate. In effect, a clinical sample is not a random subset of the population and therefore rates of comorbidity reported in clinical samples should be treated with caution. This caveat is especially relevant regarding the prevalence of irritability in ASD. Largely due to the uncertain nosological status of irritability (see Section 1.2.2), it has not yet been investigated in an epidemiological sample; therefore research on irritability in ASD has so far been conducted using clinical or referred samples.

Nosological reasons. Angold and colleagues (Angold, 1988; Angold, Costello, & Erkanli, 1999) and Maj (2005) contrasted comorbidity research in psychiatry to that in other branches of medicine, where the separate "disease categories" are relatively better delineated. They pointed out that psychiatric disorders are defined in terms of deviation from "normality", and as such the consensus about the correct threshold for what constitutes "abnormality" is harder to achieve. Consequently, the co-occurrence of multiple psychiatric disorders could reflect inadequately demarcated classification criteria for these disorders, rather than their true comorbidity. The lack of precision is reflected, for example, in the high prevalence of "Not Otherwise Specified" variants of diagnoses in clinical settings, which provide a diagnostic home for those individuals who do not meet strict criteria for a given disorder (Regier, Narrow, Kuhl, & Kupfer, 2009). In addition, as mentioned in the previous chapter, classification of psychiatric disorders changes with diagnostic manual editions, reflected in changes of criteria for ASD, introduction of new diagnostic categories (e.g., DMDD), and re-classification of existing diagnoses (e.g., OCD).

A second problem is the presence of definitional overlap, where the same symptom is included in the diagnostic criteria for another (Caron & Rutter, 1991). This can lead a clinician to misattribute the symptom to just one diagnosis, so-called "diagnostic overshadowing", resulting in underidentification of a potential comorbidity – particularly if the comorbid condition is considered less significant than the primary diagnosis, e.g. in case of developmental disability (J. Mason & Scior, 2004). Alternatively, a clinician may incorrectly diagnose two separate disorders when a single diagnosis is more appropriate. If this error occurred in the context of an epidemiological study, e.g. due to inexperienced raters mistakenly diagnosing anxiety in young people with ASD, the comorbidity rates could be inflated due to ascertainment bias. There is a number of symptoms and behaviours that feature in the presentation of ASD that may resemble those found in other disorders. As mentioned previously, restrictive interests and repetitive behaviours, characteristic of ASD, may resemble obsessions and compulsions found in OCD (e.g., repetitive asking, ordering toys in a room, continual thoughts that do not go away) (Zandt, Prior, & Kyrios, 2007). It may be possible to distinguish these seemingly identical presentations of ASD and OCD by examining whether the thoughts and behaviours are ego-syntonic or ego-dystonic, respectively. Likewise, compulsions in OCD are by definition linked with a specific obsession (e.g., fear of contamination or the need for things to be "just right"), which may not be the case in ASD (Wood & Gadow, 2010). Another example for the apparent overlap between ASD and anxiety is reluctant participation in social situations, which could be a manifestation of avoidance in social anxiety disorder or ASD-specific lack of interest in the social situation. Also, while speech and communication difficulties are found in both ASD and social anxiety, the quality of impairments is different. Communication deficits in ASD are a defining feature of the disorder that, unlike in social anxiety, persist across contexts and situations, and are characterised by several specific impairments e.g. pronoun reversal and echolalia (Wood & Gadow, 2010). These apparent similarities underscore the importance of a thorough diagnostic assessment that should consider possibly different motivations behind similar observed behaviours. As I will discuss below, research into the underlying pathophysiology may also help differentiate cases of true comorbidity from mere epiphenomena.

2.1.2 Chance comorbidity

An important consideration when studying comorbidity is to ensure that the rate at which the disorders co-occur is not simply what would be expected by chance. The rate of chance comorbidity can be estimated by multiplying the respective prevalences (base rates) of each disorder in the general population (Caron & Rutter, 1991). For the reasons outlined above, a methodologically robust method of obtaining this estimate for ASD and other disorders would

be to use one epidemiological dataset where the prevalences of each relevant disorder were assessed. The most recent study of this kind in the UK is the British Child and Adolescent Mental Health Survey (B-CAMHS04) (H. Green, McGinnity, Meltzer, Ford, & Goodman, 2005) carried out by the Office for National Statistics in 2004. The sample comprised 7977 children and adolescents aged 5-16 years old that were representative of the general British population. ICD-10 diagnoses for all major psychiatric disorders were included. The prevalence of ASD in the B-CAMHS04 was 0.9% out of all children studied, and was higher for boys (1.4%) than for girls (0.3%). The prevalence of anxiety disorders (including separation anxiety, specific phobia, social phobia, panic, agoraphobia, PTSD, OCD, GAD and "other anxiety") was 3.3% across all children surveyed and was higher for girls (3.8%) than for boys (2.9%). Based on these prevalences, one can estimate that the rate of ASD and anxiety disorders co-occurring by chance is 0.009 x 0.033 = 0.000297, therefore approximately 0.03% for all children; and 0.04% and 0.01% for boys and girls, respectively. The B-CAMHS study reported that 16% of children with ASD surveyed in 1999 and 2004 had at least one additional diagnosis of an ICD-10 anxiety disorder. This rate is much higher than the prevalence of combined ASD and anxiety expected by chance, arguing against purely chance comorbidity. Similarly in the case of depression, with the B-CAMHS-reported base rate of 0.6% (0.8% for girls and 0.5% for boys), one would expect a 0.01% prevalence of depression in children with ASD if the comorbidity was to occur by chance (0.01% for boys, 0.002% for girls). This contrasts with the reported 1% prevalence of depression in children with ASD, again arguing against chance comorbidity. Lastly, while a study of irritability symptoms in ASD using an epidemiological sample is still lacking, the rate of ICD-10-defined ODD in the B-CAMHS04 was 3.0% for all children; 4.0% for boys, 2.0% for girls. Accordingly, if ASD and ODD were to co-occur by chance, we would expect the prevalence of combined ASD+ODD at 0.03% for all children, 0.04% for boys and 0.02% for girls. The prevalence of ODD in children with ASD was not reported in the B-CAMHS04; however Simonoff et al (2008) reported a much higher rate (28.1%) of the two disorders co-occurring in their population-derived sample.

2.1.3 Models of true comorbidity

Several non-artefactual conceptualisations of comorbidity have been proposed (Angold et al., 1999; Banaschewski, Neale, Rothenberger, & Roessner, 2007; Caron & Rutter, 1991; Neale & Kendler, 1995), each providing predictions about whether the aetiological mechanisms underlying comorbidity are shared or distinct from the mechanisms of its constituent, independent disorders. The different models of comorbidity discussed in this section are illustrated schematically in Figure 2.1 on page 57.

The ***alternate forms*** model (Neale & Kendler, 1995), also referred to as "common aetiology" (Banaschewski et al., 2007) assumes that the two comorbid conditions share pathophysiological mechanisms, and that a single pathophysiological liability that is not diagnosis-specific may produce alternative phenotypes. Consistent with this model, emerging evidence from behavioural genetics shows increased levels of anxiety in both children with ASD and their unaffected co-twins (Hallett, Ronald, et al., 2013), suggesting familial aggregation of ASD and anxiety. In addition, the effectiveness of SSRIs in alleviating some ASD symptoms as well as mood and anxiety problems (Hollander, Kaplan, Cartwright, & Reichman, 2000; Namerow, Thomas, Bostic, Prince, & Monuteaux, 2003) suggests that these disorders may share aetiological mechanisms, although it would be incorrect to assume common aetiology based solely on treatment response. It has also been suggested that the serotonin transporter (5-HTT) gene, thought to be associated with some affective disorders (Kruschwitz et al., 2014; Lasky-Su, Faraone, Glatt, & Tsuang, 2005; Lotrich & Pollock, 2004) may also play a role in ASD. However, the association between common 5-HTT gene polymorphisms and ASD failed to reach significance in meta-analyses (Huang & Santangelo, 2008; Warrier, Chee, Smith, Chakrabarti, & Baron-Cohen, 2015), although significant heterogeneity between studies was noted.

The ***additive model*** of comorbidity assumes that the comorbid condition presents with combined deficits of those seen in "pure", constituent disorders (Banaschewski et al., 2007). A 2x2 design is particularly fitting to test this model, since it allows comparisons between the comorbid group, two "pure disorder" groups and healthy controls. Emerging evidence suggests that the additive model of comorbidity may characterise the co-occurrence of ASD and psychopathic, callous-unemotional (CU) traits. Rogers and colleagues compared 18 boys with ASD and low CU traits to 10 boys with ASD and high CU on a range of ASD-specific and psychopathy-specific measures (Rogers, Viding, Blair, Frith, & Happé, 2006). While the groups shared ASD-specific impairments such as severity of ASD symptoms and theory of mind deficits, the high CU traits group displayed additional, psychopathy-specific impairments, including deficits in empathy (Blair, 2005; Seara-Cardoso, Sebastian, Viding, & Roiser, 2016) and difficulties in recognising the facial expression of sadness (Blair, Colledge, Murray, & Mitchell, 2001). In addition, while ASD traits and CU traits both showed high heritability in a large sample derived from the TEDS dataset, their underlying genetic and environmental mechanisms were largely independent (O'Nions et al., 2015), in line with the conceptualisation of ASD and CU traits as separate constructs. Evidence from ERP studies suggests the additive model may also fit the comorbidity pattern observed between ASD and ADHD. By comparing the neurophysiological markers of face processing between four groups (ASD only, ADHD only, ASD+ADHD and controls) Tye and colleagues showed that children with ASD+ADHD display combined ASD-specific gaze processing difficulties and ADHD-

specific early visual attention problems (Tye et al., 2013). This was also true for processing of emotional face stimuli, where children with combined ASD+ADHD showed both ASD-specific stimulus encoding difficulties and ADHD-specific contextual processing abnormalities (Tye, Battaglia, et al., 2014). Similar additive comorbidity effects were found for executive functioning, whereby the combined ASD+ADHD group displayed both ASD-specific conflict monitoring deficits as well as attentional and inhibitory control difficulties seen in the ADHD only group (Tye, Asherson, et al., 2014). The additive model therefore assumes that a double dissociation can be identified between the "pure" effects of the disorders that comprise the comorbid condition, and that both of these effects are present in the comorbid group. The underlying pathophysiological mechanisms of these impairments may be shared, or distinct but correlated with each other (Banaschewski et al., 2007), as illustrated in Figure 2.1.

According to the ***independent nosology*** model of comorbidity (Banaschewski et al., 2007; Caron & Rutter, 1991), the comorbid condition represents a distinct diagnostic entity or so-called "third independent disorder" (Neale & Kendler, 1995) with aetiological mechanisms different to those of the "pure" disorders. For example in case of ADHD and tic disorder, the comorbid condition is underlain by unique neurophysiological ERP markers (Yordanova, Heinrich, Kolev, & Rothenberger, 2006) and unique familial risk factors (S. E. Stewart et al., 2006) that are not shared with either "pure" condition. Emerging evidence suggests that mood dysregulation symptoms, characterised by irritable and labile mood, may present differently in youth with and without ASD. Young people with SMD show facial affect labelling deficits across a range of emotions (happy, angry, sad, and fearful) (Guyer et al., 2007; P. Kim et al., 2013; Rich et al., 2008). By contrast, young people with ASD and similarly-defined severe mood problems (SMP) did not show facial emotion labelling deficits except for recognising the expression of surprise (Simonoff et al., 2012). In addition, after controlling for the effects of IQ, severe mood problems in youth with ASD were not associated with performance on response reversal tasks (card sort and trail making) (Simonoff et al., 2012), while youth with SMD were significantly less likely than controls to maintain the correct response pattern based on probabilistic reinforcement in a response reversal paradigm (Dickstein et al., 2010). In a similar vein, attentional bias to threat, often found in anxiety disorders (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007), was not associated with anxiety symptoms in boys with ASD (Hollocks, Ozsivadjian, Matthews, Howlin, & Simonoff, 2013). The lack of association across two different dot-probe tasks used in the Hollocks et al (2013) study (emotional faces and emotional words) suggests that the finding was not measure-specific. While these studies suggest that irritability and anxiety may be underpinned by distinct mechanisms in ASD and TD youth, conclusions are limited due to methodological limitations. Evidence comes from separate studies and heterogeneity in task design and sample

characteristics (in particular the definition of SMD vs. SMP) could act as a confounder. Furthermore, the exact pattern of impairments displayed by children with ASD (compared to TD) on these cognitive tasks is not yet sufficiently clear, with studies bringing mixed results. For instance, a meta-analysis of emotion labelling in ASD showed impaired labelling of some, but not all emotions (Uljarevic & Hamilton, 2012), while some (Corbett, Constantine, Hendren, Rocke, & Ozonoff, 2009; Geurts, Verté, Oosterlaan, Roeyers, & Sergeant, 2004; Pellicano, Murray, Durkin, & Maley, 2006) but not all studies (Dichter et al., 2010; Hill & Bird, 2006; Poljac et al., 2010) reported differences between youth with ASD and controls on cognitive set shifting. With regards to attentional threat bias in ASD, studies consistently do not find ASD vs. TD differences on behavioural task performance (Hollocks et al., 2013; May, Cornish, & Rinehart, 2015; Monk et al., 2010), but emerging evidence suggests that the groups may differ in terms of amygdala recruitment during the task (Monk et al., 2010). Banaschewski and colleagues (2007) suggested that a 2x2 design would again be appropriate to investigate a possible case of independent nosology. In particular, an interaction between the effects of two disorders may indicate that the comorbid condition is underpinned by distinct aetiological mechanisms, although it is not possible to fully distinguish between qualitative and quantitative group differences based on the presence of a statistically significant interaction alone (Banaschewski et al., 2007).

The ***multiformity*** model of comorbidity (Neale & Kendler, 1995) proposes that comorbid condition B is an epiphenomenon, or symptomatic phenocopy (Banaschewski et al., 2007) of disorder A. In this case, symptoms of disorder B arise exclusively due to liability for condition A and are unrelated to the independent risk factors for disorder B. According to this model, anxiety symptoms in ASD would be expected to be fully explained by the risk factors for ASD, and greater severity of anxiety symptoms would correspond to greater severity of ASD. However, both of these assumptions are inconsistent with a recent study that examined the discriminant and convergent validity of anxiety symptoms in 88 children with ASD. Renno and Wood (2013) used structural equation modelling to show that anxiety and ASD symptoms were separate constructs (with anxiety clearly differentiated from ASD severity) based on reports from the children themselves, their parents, and diagnostic interviewers alike. Similarly, the internal construct validity of GAD, ODD, and depression was also supported by parent- and teacher-reports of psychiatric symptoms in a large sample (n=498) of referred children with ASD (Lecavalier, Gadow, DeVincent, & Edwards, 2009); and Simonoff et al (2008) did not find an association between anxiety severity and the number of ASD symptoms in their study. While some studies suggest that specific domains of ASD symptoms correlate with specific anxiety symptoms (Hallett, Ronald, et al., 2013; Stratis & Lecavalier, 2013), and that ASD severity may be associated with atypical, as opposed to traditional, manifestations

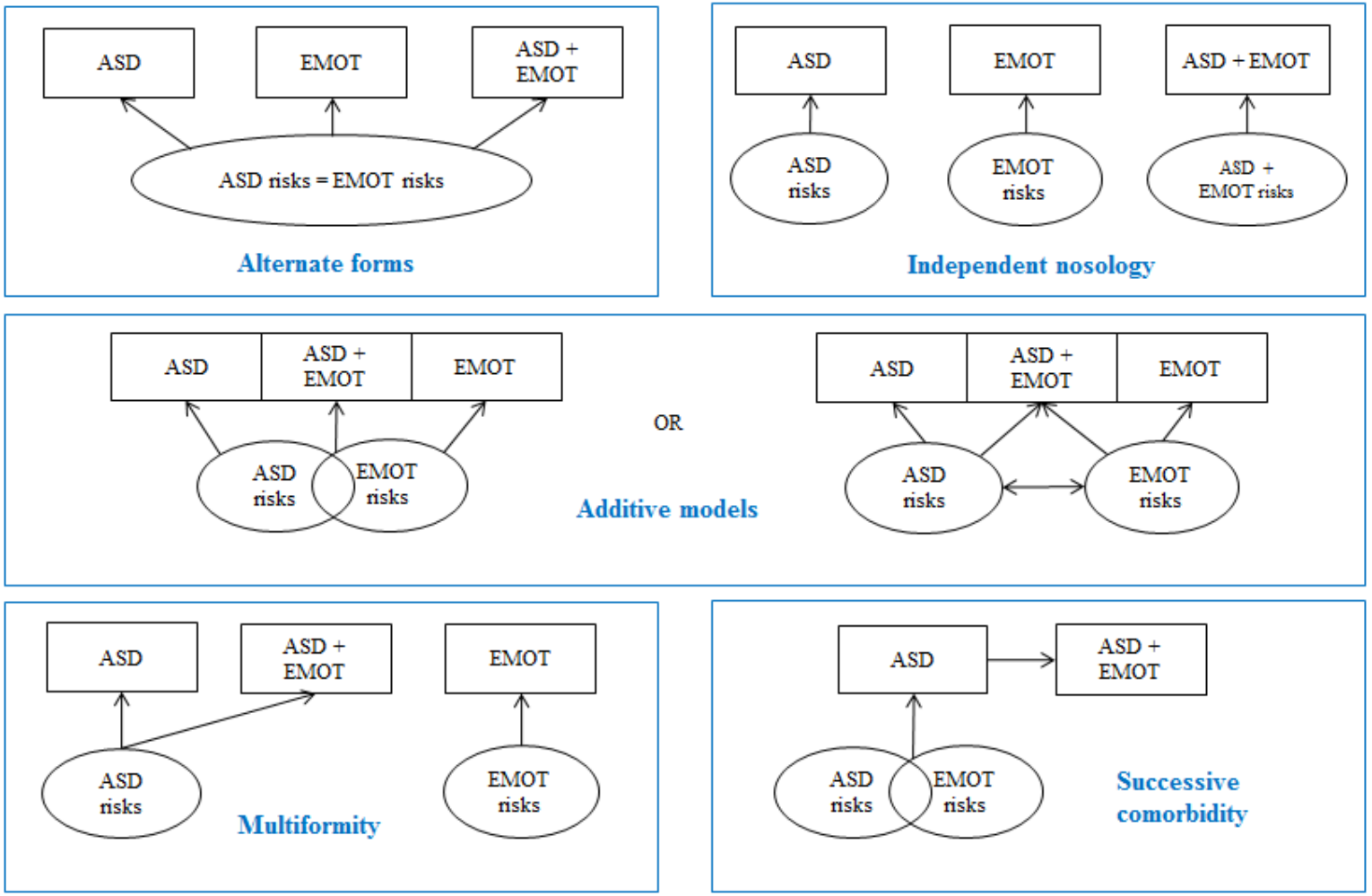
of anxiety (Kerns et al., 2014), the multiformity model is unlikely to fully account for the presence of emotional symptoms in young people with ASD.

Above I described instances where two disorders co-occur at the same time, i.e. the case of concurrent comorbidity. It is also worth mentioning ***sequential or successive comorbidity*** (Angold et al., 1999) whereby having one disorder increases the likelihood of another disorder emerging later in time, via risk factors that are shared between the two disorders (Caron & Rutter, 1991). A well-established example of successive comorbidity is the longitudinal link between ODD and later depression (Loth, Drabick, Leibenluft, & Hulvershorn, 2014; Rowe, Costello, Angold, Copeland, & Maughan, 2010; Stringaris & Goodman, 2009a) that is specifically associated with irritability (Stringaris & Goodman, 2009a), possibly due to shared genetic risk factors (Stringaris, Zavos, et al., 2012). In the case of persistent, neurodevelopmental disorders such as ASD, it would be assumed that ASD acts as a pre-existing condition that creates increased risk for another disorder. The mechanism by which ASD may increase the liability for another psychiatric condition could be due to aberrant development of the social neural systems (Damiano et al., 2015) that may have downstream effects on information processing, cognitions, and behaviour. Impaired emotion regulation has been proposed as one potential mechanism of successive comorbidity in ASD, although the accounts have been largely theoretical (Mazefsky et al., 2013; Mazefsky, Pelphrey, & Dahl, 2012; S. W. White et al., 2014). Emerging evidence suggests that young people with ASD use maladaptive emotion regulation strategies (e.g., rumination) more often than TD youth (Mazefsky, Borue, Day, & Minshew, 2014; Samson et al., 2015) and that greater endorsement of these maladaptive strategies is associated with higher rates of psychopathology, in particular self-reported internalising symptoms (Mazefsky et al., 2014). Combined with the well-known association between maladaptive emotion regulation strategies and emergence of internalising disorders in the TD population (for a meta-analysis, see Aldao, Nolen-Hoeksema, & Schweizer, 2010), impaired emotion regulation provides a plausible explanation for the high rates of comorbidity between ASD and emotional disorders. However, further, longitudinal research is needed to investigate whether emotional regulation acts as a mechanism underlying their successive comorbidity. As proposed by Caron and Rutter (1991), to test for successive comorbidity one should investigate whether disorder "B" and comorbid A+B share risk factors, and whether it is disorder "A" that generates these risk factors. Accordingly, Green et al (2012) showed that sensory over-responsivity in toddlers with ASD predicted anxiety symptoms one year later, but anxiety did not predict sensory over-responsivity.

Alternative approaches to investigating comorbidity. In this section I have focused on traditional models of comorbidity that have been put forward to explain the co-occurrence of distinct diagnostic categories in a single individual. Recently, neuroscience-based

approaches such as the Research Domain Criteria initiative (RDoC; http://www.nimh.nih.gov/research-funding/rdoc.shtml) (Insel et al., 2010) have been put forward to investigate processes that cut across diagnostic boundaries. Within the realm of the RDoC, comorbidity may occur because several different diagnoses are characterised by abnormalities in the same, interconnected neural circuits (Joormann & Goodman, 2014). Dimensional assessment of behaviours and the mechanisms underlying them may prove especially useful in elucidating the reasons for co-occurrence of symptoms that do not meet strict diagnostic criteria (e.g., subthreshold depressive symptoms or ASD traits).

Figure 2.1. Possible models of comorbidity between ASD and emotional problems (EMOT). Rectangles depict phenotypes; circles represent risk factors / underlying pathophysiological mechanisms. See main text for a detailed explanation.



2.2 Reward processing

As mentioned in the previous section, the co-occurrence of two or more disorders presents an opportunity for investigating the reasons for the overlap, which may have important nosological and clinical implications. In ASD, investigating the mechanisms underlying comorbidity may help understand whether the co-occurring disorders share their pathophysiology with the TD population, or whether the comorbid condition is somehow altered when it presents in the context of ASD. This is especially important as the rates of mood and anxiety disorders are reportedly higher in the ASD compared to TD population.

In this section, I introduce reward processing as one possible mechanism underlying the comorbidity between ASD and affective disorders, including symptoms of anxiety, irritability, and depression. Aberrations in the processing of reward have been reported in all these conditions, and hence may underlie the co-occurrence of these disorders in a single individual.

2.2.1 What is reward?

Before discussing reward processing in affective disorders and ASD, it is necessary to define some key concepts. A rewarding stimulus is one that, through the activation of a distributed and integrated set of neural systems, leads to a hedonic reaction ("liking") and generates motivation to approach the stimulus ("wanting") (Berridge & Kringelbach, 2008; Richards, Plate, & Ernst, 2013). Primary rewards – such as food, water, pleasant sounds or sexual stimuli – reinforce behaviours without having to be learned, whereas secondary rewards, e.g. money or power, gain reward value through the learning of stimulus-reward associations. As such, adequately encoding reward-related information and relating it to action is essential for adaptive behaviour and survival, as well as subjective pleasure and well-being.

Neuroscience research distinguishes between two main phases of reward processing: reward anticipation and consummation/feedback. Reward anticipation refers to the appetitive or motivational processes stimulated by the detection of an incentive, whereas reward consummation occurs after the reward is received. Both animal (Robbins & Everitt, 1996) and human studies (Diekhof, Kaps, Falkai, & Gruber, 2012; Knutson, Fong, Adams, Varner, & Hommer, 2001; Liu, Hairston, Schrier, & Fan, 2011) suggest different patterns of neural activation underlying these two phases of reward processing. In addition, one can distinguish between different reward valences, i.e. the positive states associated with reward receipt vs. negative states associated with loss or reward omission.

2.2.2 The neural circuitry of reward processing

Early evidence for the involvement of specific brain regions in reward processing comes from studies of non-human primates. Animal research allows the investigation of neural networks via targeted lesions and by studying single neuron firing rates. Studies of the macaque monkey showed that neurons in the ventral striatum respond consistently to rewarding stimuli, both during reward consummation (Apicella, Ljungberg, Scarnati, & Schultz, 1991) and when the monkey is expecting a reward based on its predictable delivery pattern (Schultz, Apicella, Scarnati, & Ljungberg, 1992). In their behavioural conditioning study, Schultz et al (1992) studied firing rates of the macaques' striatal neurons in a delayed go-no-go task, where correct performance (pressing a button in response to light of a particular colour) was reinforced by a drop of fruit juice. They demonstrated phasic increases in firing rates of striatal dopamine neurons that preceded reward delivery and disappeared when the reward failed to appear after a few trials. Later studies showed differential activity in the monkey ventral striatum with even minute differences in reward magnitude (e.g., <0.1 ml differences in concentration of the fruit juice) (Cromwell & Schultz, 2003), suggesting ventral striatal involvement in encoding reward value. The orbitofrontal cortex (OFC) is another region whose involvement in reward processing has been widely documented in animal studies. As a structure that receives somatosensory, visual and olfactory inputs, the OFC has been shown to take part in representing the reward value of primary reinforcers such as touch, smell and taste. For example, Rolls (1989) demonstrated that the OFC neurons of a monkey respond to the taste of glucose, and that the neuronal response decreases to zero when the monkey has been fed to satiety with food of the same taste. This was different to the sustained response of neurons in the primary taste cortex that were not modulated by satiety, suggesting that neurons in the monkey OFC specifically encode the reward value of taste, rather than the detection of taste itself. In addition, targeted lesions of the OFC in non-human primates have been shown to lead to behavioural inflexibility and perseveration errors on reward reversal tasks (Clarke, Robbins, & Roberts, 2008; Izquierdo, Suda, & Murray, 2004), suggesting that the OFC is involved in updating stimulus-reward associations based on changes in reward contingencies.

In humans, the neural circuitry of reward processing has been predominantly studied using fMRI reward tasks. One of the most widely used is the monetary incentive delay (MID) task developed by Knutson and colleagues (Knutson, Westdorp, Kaiser, & Hommer, 2000) to measure BOLD signal changes during reward anticipation and feedback. In this event-related task, the participant first sees a visual cue that s/he has previously learned to associate with a reward of a given magnitude (e.g., a small or large amount of money/points). Next, a target appears on the screen and the participant needs to press a button corresponding to the target's location as soon as possible. At the end of each trial, the participant receives on-screen feedback indicating whether they responded successfully, how much money/points they won on this trial and the cumulative amount of money/points won on the task so far. BOLD responses are collected

during anticipation and feedback phases of the task, and studies distinguish between positive feedback (where the participant responds successfully and receives the reward), reward omission (when the reward fails to appear despite correct performance) and loss conditions (where an amount of money is deducted following unsuccessful performance).

Consistent with animal studies summarised above, fMRI studies in humans also implicate the striatum and OFC as core components of the reward processing circuitry. The striatum can be subdivided into ventral and dorsal subregions. Both OFC and ventral striatum, which comprises the nucleus accumbens (NAcc) and ventral parts of the caudate and putamen (Haber & McFarland, 1999), receive dopaminergic projections via the mesolimbic pathway (Oades & Halliday, 1987). Recent meta-analyses of human neuroimaging reward studies suggest that while the OFC is more commonly activated during reward consummation (obtaining a reward increases activation in the medial and orbitofrontal regions of the brain), the ventral striatum is implicated in both reward anticipation and feedback (Diekhof et al., 2012; Liu et al., 2011). The ventral striatum displays a characteristic response pattern during human reward processing, whereby its activation increases when anticipating rewards, but decreases when the anticipated reward is not subsequently obtained (Knutson, Fong, et al., 2001; Knutson, Fong, Bennett, Adams, & Hommer, 2003). In addition, the ventral striatal response to obtaining a reward is often most pronounced when the reward occurs unpredictably (Berns, McClure, Pagnoni, & Montague, 2001; Cohen, Young, Baek, Kessler, & Ranganath, 2005; Yacubian et al., 2006); suggesting that the role of the ventral striatum during reward consummation may be best understood in terms of tracking the reward prediction error (McClure, Berns, & Montague, 2003; O'Doherty, Dayan, Friston, Critchley, & Dolan, 2003). Reward prediction error signalling refers to the detection of a difference between the expected and the received amount of reward, which allows us to update future reward expectancies for a given stimulus (Rescorla & Wagner, 1972). By contrast, the involvement of orbitofrontal and medial portions of the PFC during reward consummation has been proposed to reflect higher-order value-based decisions (Diekhof et al., 2012; Noonan, Kolling, Walton, & Rushworth, 2012; Sescousse, Redoute, & Dreher, 2010) whereby the medial PFC integrates stimulus value across contexts, e.g. by showing a heightened response to a desirably high price in the context of selling, but a lower response to the same high price in the context of buying (Knutson & Greer, 2008).

Several other brain regions have been implicated in reward processing. Dorsal putamen, which is connected to the sensorimotor cortices (Delgado, 2007), has been implicated in relating the salience of a reward cue to action and guiding the subsequent motor response (Alexander & Crutcher, 1990; Haruno & Kawato, 2006). Activation in the insula is often reported during reward anticipation (Ernst et al., 2004; Knutson, Fong, et al., 2001). Combined with its purported role in encoding uncertainty and risk prediction learning (Critchley, Mathias, & Dolan, 2001; Preuschoff, Quartz, & Bossaerts, 2008), the insula has been proposed to form part of the aversion/avoidance reward network (Richards et al., 2013). Finally, several cortical regions have been implicated in

"top-down", regulatory functions during reward processing, including the ACC engaged during error monitoring, conflict detection and resolution (Bissonette & Roesch, 2016; Rushworth, Noonan, Boorman, Walton, & Behrens, 2011), dlPFC involved in working memory (Fletcher & Henson, 2001), and IFG guiding response inhibition (Aron, Robbins, & Poldrack, 2014). A recent meta-analysis demonstrated that these "cognitive control" regions are more likely to be recruited during reward processing in adults compared to adolescents (M. H. Silverman, Jedd, & Luciana, 2015).

2.2.3 Reward processing in ASD

Several researchers have recently discussed reward processing aberrations in ASD in the context of the social motivation theory (Dichter & Adolphs, 2012; Hernandez, Rudie, Green, Bookheimer, & Dapretto, 2015). The theory proposes that decreased motivation to attend to social cues plays a central role in ASD, leading to fewer interactions with the social world and thus limiting social learning (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012; Dawson, Webb, & McPartland, 2005).

Accordingly, social deficits are thought to persist across development, whereby even very young children with ASD fail to orient to social stimuli and show deficits in shared attention (Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998), while adolescents (Chevallier, Grèzes, Molesworth, Berthoz, & Happé, 2011) and adults with ASD (Berthoz, Lalanne, Crane, & Hill, 2013) report experiencing less pleasure from social interactions, compared to typically developing controls. Consistent with the social motivation theory, children with ASD (but not TD children) took longer to respond to social compared to monetary rewards in an adapted version of the MID task (Demurie, Roeyers, Baeyens, & Sonuga-Barke, 2011) and displayed aberrant processing of social but not non-social stimuli during reward anticipation in an ERP study (Stavropoulos & Carver, 2014). Moreover, in fMRI studies of reward processing, children with ASD showed decreased striatal activity during social reward feedback (Delmonte et al., 2012) and social rewarded learning (Choi et al., 2015; Scott-Van Zeeland, Dapretto, Ghahremani, Poldrack, & Bookheimer, 2010). Nonetheless, other studies also reported reduced striatal activation during monetary reward processing in children (Damiano et al., 2015; Kohls et al., 2013) and adults with ASD (Dichter, Felder, et al., 2012; Dichter, Richey, Rittenberg, Sabatino, & Bodfish, 2012; Richey et al., 2014), suggesting domain-general reward circuitry hypoactivation in this population (see Table 2.1 for a summary of previous fMRI studies that compared reward processing in children and young people with ASD to that of TD controls).

Importantly however, reduced reward responsiveness in ASD seemingly does not apply to stimuli related to circumscribed interests. These stimuli were shown to capture visual attention of children with ASD during passive viewing (Sasson, Turner-Brown, Holtzclaw, Lam, &

Bodfish, 2008) and attained a higher reward value (Dichter, Felder, et al., 2012; Watson et al., 2015) in children with ASD compared to controls. In a carefully designed fMRI study, Cascio and colleagues (2014) contrasted neural responses to pictures of one's own interests (e.g., trains, horses) with pictures depicting other children's interests. Participants were children with ASD and age- and IQ-matched, typically developing controls with strong interests or hobbies. Cascio et al demonstrated that while both groups displayed comparable activity in the reward circuitry when viewing pictures depicting their own interests, children with ASD (but not controls) showed reduced activation in the insula and ACC when viewing pictures of other children's interests.

Taken together, evidence suggests that the differences in neural substrates of reward processing between individuals with ASD and TD controls may in some cases be modulated by the salience of reward that, in children with ASD, biases attention towards idiosyncratic stimuli. Specific features of ASD may be differentially associated with reward processing in this population: decreased motivation to engage in social reciprocity may manifest in hypoactivation of the reward circuitry during social reward processing, while ASD-specific restrictive interests may lead to heightened motivation and enhanced reward-related circuitry activation in response to idiosyncratic incentives.

2.2.4 Reward processing in disorders that co-occur with ASD

The key question of comorbidity has not been investigated in relation to reward processing in youth with ASD. Indeed, seven of the eight published fMRI studies that investigated reward processing in children and adolescents with ASD excluded participants with psychiatric comorbidities from their samples, while one did not specify whether participants with comorbidities were included (see Table 2.1). Therefore, participants with ASD who took part in these studies are not representative of the wider ASD youth population where close to 70% have at least one co-occurring psychiatric disorder (Simonoff et al., 2008). In adult literature, Richey et al (2014) used fMRI to compare the neural correlates of reward processing between 16 individuals with ASD, 15 patients with social anxiety disorder, and 19 controls. Participants with ASD showed reduced NAcc activation during monetary, but not social reward anticipation relative to those with social anxiety, suggesting that reward sensitivity to specific reward types may differentiate ASD from social anxiety. However, this study did not report comorbidity rates in the ASD sample, limiting conclusions that can be drawn about ASD-specific contributions to aberrant reward processing. Investigating the relative contributions of ASD symptoms and co-occurring disorders on reward processing may shed light on the models of comorbidity between these disorders and the pathophysiological mechanisms underlying the comorbid condition, which has important clinical implications. Below I present a short summary of reward processing aberrations in disorders and symptoms that often co-occur with ASD.

Anxiety. Studies of children with anxiety disorders or behavioural inhibition often find increased striatal engagement during reward anticipation (Bar-Haim et al., 2009; Guyer et al., 2006), enhanced ACC activation following error commission (Ladouceur, Dahl, Birmaher, Axelson, & Ryan, 2006) and increased medial PFC activation following negative reward feedback (Helfinstein et al., 2011). Enhanced striatal activation during reward anticipation has been proposed to reflect a heightened concern about making errors on the task and desire to avoid failure (Guyer et al., 2006), consistent with an adult study where striatal recruitment was higher during punishment avoidance vs. reward anticipation trials in participants with social anxiety disorder relative to controls (Cremers, Veer, Spinhoven, Rombouts, & Roelofs, 2014). Interestingly, striatal hyperactivation during reward anticipation was found to be more pronounced in paediatric social anxiety than in generalised anxiety (GAD), while children with generalised anxiety displayed a unique pattern of increased putamen activation when anticipating potential gains but not losses (Guyer, Choate, Detloff, et al., 2012), suggesting that different types of anxiety can differentially impact on the neural substrates of reward processing. With regards to reward feedback, an ERP study of 390 children from a community sample found different associations between social anxiety vs. GAD symptoms and reactivity to monetary gains, with social anxiety being associated with heightened sensitivity to rewards (Kessel, Kujawa, Hajcak Proudfit, & Klein, 2015).

Depression. In contrast to findings in anxiety literature, young people with depression and subclinical depressive symptoms display reduced striatal activation during reward anticipation relative to healthy controls (Forbes et al., 2006; Forbes et al., 2009), suggesting a decreased motivation to seek rewarding stimuli. Consistent with this explanation, a recent fMRI study using the MID task found that the pattern of decreased ventral striatal activation when anticipating reward is specifically associated with anhedonia, rather than low mood, component of adolescent depression (Stringaris et al., 2015). Decreased reward seeking in children with depression is also evident behaviourally, whereby those with depression (but not anxiety or externalising disorders) are less likely to choose large rewards in a high-probability rewarding scenarios (Forbes, Shaw, & Dahl, 2007).

Externalising symptoms. Youth with behavioural difficulties, in particular ODD symptoms and irritability, often show aberrant responses when rewards fail to appear, perhaps due to frustrative nature of negative outcomes (Bjork, Chen, Smith, & Hommer, 2010; Deveney et al., 2013). This is coupled with increased risk-taking that may manifest in disproportionately large motivation to seek rewards even when the probability of a potential loss/punishment is high (Matthys, van Goozen, Snoek, & van Engeland, 2004). Relative to healthy controls, young people with ODD/CD display less activation in the OFC and caudate in the early stages of reward learning (early vs. late trials) (Finger et al., 2011). They also show reduced caudate response to positive reward feedback, but increased caudate response to negative feedback (S. F. White et al., 2013). This suggests that these youth may have specific difficulties with encoding the value of

rewards based on prediction error, and could explain why young people with disruptive behaviour problems are likely to make disadvantageous choices (Viding & Seara-Cardoso, 2013).

The relative contribution of ASD traits and comorbidities to aberrant reward processing has not been tested directly in young people with ASD. Limited evidence comes from a study that compared risk-taking between children with ASD and TD controls. In their study, South and colleagues (2011) asked participants to select a number of "pumps" (ranging from 1 to 128) to pump each of 30 balloons that appeared on the screen. While the participants could win 1 point for each additional pump, the balloons had a varying explosion point and selecting too many pumps would result in the balloon bursting and losing all points for a given balloon. While there were no differences in overall risk-taking and reaction times between the two groups, the moderators of risk-taking differed. Risk-taking was predicted by parent-reported anxiety symptoms in children with ASD, but not controls. Within the control group only, risk-taking correlated with the behavioural activation system (BAS). The authors proposed that task performance in the ASD group may have been motivated by fear of failure, compared to reward sensitivity in controls. This explanation is consistent with Guyer et al's (2006) hypothesis of failure avoidance in children with anxiety that manifests in enhanced striatal activation when anticipating reward. Nevertheless, neural responses were not investigated in the South et al (2011) study and it remains to be tested whether anxiety symptoms upregulate striatal and possibly insular (Richards et al., 2013) activity during reward anticipation in children with ASD. At present, the extent to which reward circuitry disruption in youth with ASD is related to emotional vs. ASD-specific symptoms remains unclear.

Table 2.1. fMRI studies that compared reward processing in children and young people (mean age < 18 years) with ASD to TD controls.

Reference	ASD n (males)	TD n	ASD age (years)	TD age (years)	Reward task	Comorbidities assessed?	Main results in ASD group (vs. TD)	Conclusions
Cascio et al (2012)	17 (17)	18 (17)	12.8 ± 2.5	13.2 ± 3.4	Primary. Passively viewed pictures of high-calorie foods after fasting.	No (exclusion criterion)	↑insula and ACC to food pictures	Enhanced neural response to primary rewards in some areas, but other frontostriatal activations similar to TD.
Cascio et al (2014)	19 (?)	18 (?)	12.6 ± 2.5	13.1 ± 3.4	Passively viewed pictures of own vs. other's interests.	No (exclusion criterion)	↑left insula and ACC when viewing own vs. others' interests. Insula activation positively correlated with parent-reported interference due to restricted interests.	Enhanced neural response to own interests in ASD.
Chantiluke et al (2015)	18 (18)	21 (21)	15.1 ± 1.9	13.7 ± 2.6	Reward reversal learning. Acute effects of placebo vs. fluoxetine.	No (exclusion criterion)	↓medial PFC and precuneus under placebo. Under fluoxetine: medial PFC activation up-regulated and normalised.	Underactivation of medial PFC during reward reversal learning may be due to underlying serotonin abnormalities.
Choi et al (2015)	27 (?)	12 (?)	9.9 ± 2.5	9.1 ± 1.6	Social reward learning.	No (exclusion criterion)	↓right: dlPFC, OFC; ↑right: parahippocampal gyrus, STG	Reduced frontal activation during social reward learning.
Damiano et al (2015)	24 (23)	21 (17)	14.4 ± 3.2	14.3 ± 3.0	Social and monetary MID; avoiding reward loss.	?	↓right caudate during monetary anticipation. ↓right putamen/NAcc, left amygdala, bilateral insula, right IFG, right ACC during sad face anticipation. No differences during loss feedback.	Reduced frontostriatal responses to social and non-social negative reinforcement in ASD.
Delmonte et al (2012)	21 (21)	21 (21)	17.6 ± 3.5	17.0 ±3.4	Social and monetary MID.	No (exclusion criterion)	No group differences during anticipation. During outcome: ↓left dorsal striatum to social rewards but not monetary.	Reward circuitry hypoactivation to social but not monetary rewards in young people with ASD.
Kohls et al (2013)	15 (15)	17 (17)	14.6 ± 3.3	13.9 ± 3.0	Go/no-go task with social and monetary rewards.	No (exclusion criterion)	↓midbrain, thalamus, amygdala and ACC to both rewards; ↓NAcc to monetary, but not social reward.	Domain-general reward circuitry hypoactivation in ASD.
Scott-Van Zeeland et al (2010)	16 (16)	16 (16)	12.4 ± 2.1	12.3 ± 1.8	Implicit learning task with social and monetary rewards.	Participants did not have comorbidities.	↓VS to both social and monetary rewards. ↓VS, ACC, ventral PFC during social reward learning.	Diminished neural responses during social reward learning in ASD, coupled with near-chance performance making stimulus-reward associations.

ACC, anterior cingulate cortex. *ASD*, autism spectrum disorder. *(dl)PFC*, (dorsolateral) prefrontal cortex. *IFG*, inferior frontal gyrus. *MID*, monetary incentive delay. *NAcc*, nucleus accumbens. *OFC*, orbitofrontal cortex. *TD*, typically developing. *STG*, superior temporal gyrus. *VS*, ventral striatum. ? = not specified. ↑, increased. ↓, reduced. Partially adapted from Dichter (2012).

2.3 Physiological responsiveness to stress

A recent systematic review of physiological stress responsiveness found that in 66.7% (n=12) of the studies, individuals with ASD responded to social stressors differently to TD people (Lydon et al., 2014). However, the authors noted that outcomes were inconsistent, even for studies using similar paradigms, and these inconsistencies persisted even when a subset of high-quality studies were examined separately. One possibility is that comorbid conditions such as irritability and anxiety may have contributed to the reported inconsistencies. Indeed, some have suggested that irritability may be associated with how individuals with ASD respond to stress (Groden, Cautela, Prince, & Berryman, 1994), although this association has not yet been studied.

In this section, I review the literature on stress responsiveness in both TD and ASD populations. A particular emphasis will be put on paradigms that investigated stress responses in social situations. I will also discuss how fear and anger reactions to stress may have different physiological underpinnings, and how this may relate to inconsistent findings in the ASD population where both anxiety and irritability are highly prevalent.

2.3.1 The biological mechanisms of stress response

Physiological responsiveness to stress has been studied extensively since the early twentieth century, including the initial "fight or flight" response characterised by Cannon (1929), as well as the resulting cascade of adaptive, regulatory responses (Selye, 1950). When faced with a threat, the body reacts by mobilising two major response systems, the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis.

The instantaneous reaction of the body when faced with a stressor is governed by the ANS that is composed of two subsystems – the sympathetic and parasympathetic branches. The sympathetic nervous system is associated with the "fight or flight" response and promotes increased arousal and mobilization of the body for action, leading to physiological reactions such as increased heart rate (HR), blood pressure and breathing rate, and the release of catecholamines (e.g. noradrenaline). High levels of catecholamines induce the production of pro-inflammatory cytokines which enhance the body's immune response when faced with an acute stressor (Dhabhar, 2002). The parasympathetic nervous system controls the return of the body back to homeostasis, promoting withdrawal of the sympathetic nervous system and inhibiting the pro-inflammatory response. Since the parasympathetic nervous system acts via the vagus nerve, it is often referred to as the 'vagal brake' (Porges, 1995).

Slower, neuroendocrine responses to stress occur following the activation of the HPA axis by the limbic system and peripheral cytokines. The HPA axis involves a cascade of

biochemical reactions. First, the corticotropin releasing hormone (CRH) is secreted from the paraventricular nucleus of the hypothalamus, which induces the release of the adrenocorticotropin hormone (ACTH) from the pituitary gland. ACTH, in turn, stimulates the secretion of cortisol from the adrenal cortex, and the levels of circulating cortisol peak at around 20 minutes post-stressor (Romanczyk & Gillis, 2006). The HPA axis operates via a negative feedback loop, whereby the duration of stress response is limited by cortisol's inhibitory effect on receptors in the hypothalamus and pituitary gland, which suppresses further release of CRH and ACTH. Therefore, while endocrine activity is elevated during stress onset, it reduces with time.

Importantly, while the HPA axis generally functions in the above-mentioned fashion in response to acute stress, prolonged exposure to stress has been associated with reduced HPA axis activity (G. E. Miller, Chen, & Zhou, 2007). For example, hypocortisolemia is often found in patients with PTSD, a condition characterised by recurrent flashbacks and distressing memories of a traumatic event (Yehuda, 2001; Yehuda, Resnick, Kahana, & Giller, 1993). Likewise, using a large, longitudinal dataset of 351 adolescents at risk of depression, Booij and colleagues showed that while recent-onset depressive symptoms were associated with an increased cortisol response to experimentally-induced stress, persistent and recurrent depression predicted blunted cortisol stress response (Booij, Bouma, de Jonge, Ormel, & Oldehinkel, 2013). Lower cortisol reactivity was also reported in children who experienced harsh parenting or maltreatment (Danese & McEwen, 2012) and the level of this physiological blunting was found to be directly proportional to the number of stressful life events reported in the previous year (Jaffee et al., 2015). Evidence therefore suggests that the reactivity of the HPA axis differs as a function of stress chronicity, and can be observed in a variety of diagnostic contexts. Theorists have proposed several explanations for the blunting of cortisol response under chronic stress; some argued that the dampened cortisol response may reflect withdrawal and disengagement when faced with uncontrollable stress (Henry, 1993; J. W. Mason et al., 2001), while others suggested that blunted physiological reactivity may be adaptive and promote resilience to ongoing stress (Del Giudice, Ellis, & Shirtcliff, 2011; Gunnar & Vazquez, 2001).

2.3.2 Methods of studying physiological stress responses

The ANS response to stress is usually measured by mean HR and heart rate variability (HRV), defined as the variation in the time interval between individual heartbeats. HRV provides a more detailed index of the dynamics of ANS stress response, because it allows investigation of the balance between the sympathetic and parasympathetic branches of the ANS. While parasympathetic activity is favoured at rest, sympathetic input dominates in response to a stressor, leading to increased HR (Thayer, Åhs, Fredrikson, Sollers Iii, & Wager, 2012). Withdrawal of the parasympathetic branch of the ANS, indexed by reduced HRV, has therefore been proposed

as a marker of stress reactivity (Porges & Byrne, 1992). Other indices of sympathetic and parasympathetic nervous systems include skin conductance response and respiratory sinus arrhythmia (RSA), respectively.

The HPA axis' activity is usually indexed by cortisol levels, collected via saliva or blood samples. Saliva samples may be better indicated in studies of stress response, so as not to confound the experiment with a potentially stressful blood-taking procedure. Basal cortisol secretion follows a diurnal rhythm, whereby cortisol levels peak early in the morning after a rapid increase within the first 30 minutes of awakening, a phenomenon called the cortisol awakening response (CAR); cortisol levels then steadily decline throughout the day (Fries, Dettenborn, & Kirschbaum, 2009; Pruessner et al., 1997). While the CAR is associated with a similar increase in cortisol levels as seen in experimentally-induced stress paradigms (Schmidt-Reinwald et al., 1999), its precise function has not yet been identified (Fries et al., 2009).

Experimental stress induction paradigms provide a controlled environment in which to examine responsiveness of the ANS and HPA axis to particular stressors. A breadth of laboratory stress paradigms exists, yet there is now meta-analytic evidence from studies in adults (Dickerson & Kemeny, 2004) and children (Gunnar, Talge, & Herrera, 2009) suggesting that only paradigms that include a social component (e.g. public exposure) or evoke negative self-referent emotions (e.g. fear of negative social evaluation, shame) reliably induce cortisol changes. The Trier Social Stress Test (TSST) (Kirschbaum, Pirke, & Hellhammer, 1993) is one of the most widely used psychosocial paradigms that has been shown to reliably induce physiological stress response, as indexed by the ANS and HPA axis reactivity (Allen, Kennedy, Cryan, Dinan, & Clarke, 2014; Dickerson & Kemeny, 2004), as well as increase self-reported stress perception and anxiety (Hellhammer & Schubert, 2012). The TSST generally consists of an anticipation period followed by a test phase during which the participant is asked to deliver a presentation and perform a mental arithmetic task in front of a panel of judges. The participant is typically informed that his or her performance will be videotaped, timed, and evaluated. The task thus taps into the three elements of a successful psychosocial stress test identified by Dickerson and Kemeny (2004), namely unpredictability, uncontrollability, and social-evaluative threat.

2.3.3 Physiological stress responsiveness: effects of irritability and anxiety in the TD population

In Section 2.3.1 I described how the ANS and HPA axis respond to stress in healthy people. A discussion of stress responsiveness in youth with affective disorders, especially symptoms anxiety and irritability, is of particular importance for this thesis. However, not many studies have specifically investigated how childhood anxiety and irritability affect physiological responses to psychosocial stressors. Where appropriate, I will supplement the discussion with evidence from

related paradigms and more broadly defined internalising and externalising symptoms, as well as offer complementary evidence from adult studies.

2.3.3.1 Fear and anger responses to stress

As mentioned in Section 2.3.2, fear of negative social evaluation has been shown to reliably induce an acute release of cortisol and increased HR in experimental stress paradigms such as the TSST (Dickerson & Kemeny, 2004). However, a response to stress need not only be one of fear and withdrawal; under particular circumstances one may react to stress angrily or aggressively, as evidenced by distinct freezing vs. hostile stress behaviours of macaque monkeys that are associated with different physiological response profiles (Kalin, 1999; Kalin, Larson, Shelton, & Davidson, 1998). Similarly, in humans, the physiological effects of a stressful experience have been proposed to depend on one's subjective appraisal of the situation (Lazarus & Folkman, 1984). Evidence from adult literature suggests that anger- and fear-related responses to stress may differ in their physiological profiles. With regards to ANS reactivity, while fear and anger stress responses have both been associated with increased HR (Ekman, Levenson, & Friesen, 1983), anger responses tend to be characterised by distinctively increased diastolic blood pressure (Ax, 1953; Christie & Friedman, 2004; Sinha, Lovallo, & Parsons, 1992). Analogous differences have been found for cortisol responsiveness, although evidence is somewhat mixed. Kazen and colleagues used a modified version of the Montreal Imaging Stress Task (MIST), an experimental paradigm based on the TSST, to induce anger in healthy adults (Kazen, Kuenne, Frankenberg, & Quirin, 2012). In contrast to fear and anxiety induction studies using the TSST, the authors reported a decrease in cortisol levels following the MIST that was associated with an increase in self-reported anger (Kazen et al., 2012). Analogous cortisol results were reported in a study of self-referent anger induction in healthy young men (Herrero, Gadea, Rodriguez-Alarcon, Espert, & Salvador, 2010) and in an emotional priming study in healthy young women, where anger priming led to a decrease in cortisol levels following discrimination, whereas shame priming led to increased cortisol reactivity (Matheson & Anisman, 2009). However, the association between anger and a reduction in cortisol levels was not always replicated. Moons and colleagues (2010) used the standard TSST paradigm to induce physiological stress responses in 183 healthy adults, and collected self-reports of both anger and fear following task completion. They found that fear reactions to the TSST were associated with a decrease in cortisol, while anger reactions – with an increase in cortisol (Moons, Eisenberger, & Taylor, 2010). In a similar vein, Lupis and colleagues found that anger expressed during the TSST predicted a stronger cortisol response, albeit only in males (n=14), and only when measured with an anatomically-based facial coding system and not by self-report (Lupis, Lerman, & Wolf, 2014). While more research is required to reliably distinguish between fear and anger influences on cortisol stress responsiveness, there are several

possible explanations for the apparent inconsistency in HPA axis reactivity to experimentally-induced stress. First, self-reports of fear and anger were moderately (r=.43) correlated in the Moons et al (2010) study, making disentanglement of the two emotions difficult. Second, Moons et al (2010) collected self-reports 30 minutes after TSST completion, while in studies by Kazen et al (2012) and Herrero et al (2010) participants reported on their mood states immediately after the TSST. This methodological difference may be of importance as Hellhammer and Schubert (2012) found subjective stress responses collected during the TSST stress phase to be the most strongly related to physiological stress reactivity. Bearing these caveats in mind, existing evidence suggests a possible dissociation of fear and anger responses to stress in TD people based on physiological reactivity profiles.

2.3.3.2 Physiological stress reactivity in internalising vs. externalising disorders

Similarly to the research on fear and anger responses to stress, studies that investigated stress responsiveness in TD youth with internalising vs. externalising symptoms often reported opposite patterns of physiological reactivity between the two groups.

Boyce and colleagues investigated ANS reactivity in 122 children aged 6 to 7 following a stress-inducing paradigm that included two psychosocial, one physical, and one emotional stressor (Boyce et al., 2001). Compared to healthy control children, those with internalising symptoms displayed greater parasympathetic withdrawal in response to the stress test (i.e., a heightened ANS stress response); whereas children with externalising symptoms were less parasympathetically reactive and additionally displayed lower sympathetic arousal. This finding adds to previous reports of abnormally increased resting HR in children with separation anxiety disorder (Rogeness, Cepeda, Macedo, Fisher, & Harris, 1990) and social phobia (Schmitz, Krämer, Tuschen-Caffier, Heinrichs, & Blechert, 2011) and a decreased resting HR in children with conduct disorder (Rogeness et al., 1990) and disruptive behaviour disorder (van Goozen, Matthys, Cohen-Kettenis, Buitelaar, & van Engeland, 2000), suggesting that both resting ANS activity as well as ANS reactivity to stress may be differentially influenced by internalising vs. externalising psychopathology. However, evidence is not always consistent, with low HR (Kramer et al., 2012) and reduced parasympathetic modulation following the TSST also reported in children with social phobia (Schmitz et al., 2011). A meta-analysis of 46 studies of vagal (parasympathetic) withdrawal to stress in children found lower levels of RSA withdrawal to be associated with more externalising symptoms (Graziano & Derefinko, 2013), consistent with Boyce et al (2001). However, the meta-analysis also found that greater levels of RSA withdrawal were associated with fewer internalising symptoms, although the effect size was small (Graziano & Derefinko, 2013).

There is corresponding evidence for differential associations between HPA axis responsiveness to stress and internalising vs. externalising symptoms. Hartman and colleagues examined cortisol reactivity in response to a psychosocial stress test in 211 adolescents. The authors reported that an enhanced cortisol response during the stress test was associated with self-reported internalising symptoms, while blunted cortisol release was associated with externalising symptoms (Hartman, Hermanns, de Jong, & Ormel, 2013). Consistent with this finding, Granger and colleagues reported that out of their sample of 102 clinically-referred children aged 7 to 17, those who displayed the highest cortisol reactivity to a parent-child conflict task were characterised by high levels of internalising symptoms such as social anxiety and withdrawal (Granger, Weisz, & Kauneckis, 1994). Furthermore, significantly increased cortisol responses to a psychosocial stress test were reported in children with social anxiety disorder (van West, Claes, Sulon, & Deboutte, 2008) and separation anxiety disorder (Brand, Wilhelm, Kossowsky, Holsboer-Trachsler, & Schneider, 2011), relative to healthy controls. On the other hand, a similarly-designed study of adolescent girls with social phobia reported a statistically equal increase in cortisol levels following the TSST in their clinical sample and in healthy control girls (Martel et al., 1999). The results reported by van West et al (2008) and Martel et al (1999) appear contradictory, yet this inconsistency may be explained by sample selection and exclusion criteria. While van West et al (2008) explicitly excluded participants with ODD, CD, and ADHD, Martel et al (1999) did not mention externalising disorders among their exclusion criteria. In light of the evidence suggesting that internalising and externalising disorders may differentially affect physiological stress responses, it is possible that the relative influence of these symptoms led to Martel et al (1999) not finding cortisol hyperactivity to stress in their sample. Indeed, several studies point on decreased HPA axis reactivity to stress in youth with externalising symptoms. Apart from the association with externalising symptoms mentioned above (Hartman et al., 2013), reduced cortisol responsiveness to stress was also reported in youth with disruptive behaviour disorders (van Goozen et al., 2000), ODD (van Goozen et al., 1998), conduct disorder (Fairchild et al., 2008), and ADHD (Pinto et al., 2016). On the other hand, evidence in the opposite direction has also been reported (Hart, Burock, London, Atkins, & Bonilla-Santiago, 2005) and the association between externalising behaviours and cortisol stress responsiveness did not reach significance in a meta-analysis (Alink et al., 2008) that carefully examined possible sources of heterogeneity, including age, sex, and clinical status. However, the authors of the meta-analysis reported that most of the included studies failed to investigate comorbid behavioural and emotional symptoms, instead focusing on the relationship between cortisol stress responsiveness and a particular externalising disorder, or, alternatively, broadly defined externalising symptoms (Alink et al., 2008). Indeed, the evidence presented above suggests that internalising and externalising problems may be associated with distinct, possibly even opposite, physiological profiles; hence both should be evaluated in a physiological stress responsiveness study. Notably, in a carefully-designed study of boys with ODD/CD, cortisol decrease in response to a

psychosocial stress induction was the strongest in those who were high on externalising symptoms but low on anxiety (van Goozen et al., 1998).

2.3.4 Physiological responses to stress in youth with ASD

A variety of stress paradigms have been used in the ASD population, including studies of sensory, physical, pharmacological, social, emotional, and attention-related stressors. A discussion of studies using these various paradigms is beyond the scope of this thesis. To allow for comparisons with TD literature presented above, in this section I am going to focus on paradigms that included a social stress component. Studies that compared ANS reactivity to such stressors between youth with ASD and TD controls are summarised in Table 2.2, while studies investigating HPA axis responsiveness are presented in Table 2.3. A comprehensive discussion of physiological stress responsiveness to a wide range of stimuli and tasks in ASD is beyond the scope of this thesis, and has been considered in recent review articles on this topic (Benevides & Lane, 2013; Lydon et al., 2014; J. L. Taylor & Corbett, 2014).

2.3.4.1 Social stress responsiveness in young people with ASD

As discussed previously, the ANS plays an important role in regulating the body's response to stress. In addition, it has been suggested that activity of the ANS underlies social engagement behaviours. The Polyvagal Theory (Porges, 2001, 2007) proposes that physiological states and social engagement are tightly linked, in that the myelinated vagus nerve activity (mediated by the nucleus ambiguous) attenuates the naturally-occurring sympathetic arousal to environmental challenge when faced with social situations. This is in contrast to the action of another vagus branch that supports fight or flight behaviours in response to threat. Correct inhibition of the sympathetic activity by the myelinated vagus nerve, in turn, promotes a calm physiological state, positive affect, and social engagement. It has been suggested that disruptions in the vagal social engagement system may contribute to emotional dysregulation and social interaction difficulties seen in individuals with ASD (Porges, 2005), whereby an under-regulated physiological arousal leads to maladaptive, 'fight or flight' responses to social situations in ASD. Consistent with this view, van Hecke et al (2009) reported that children with ASD showed decreased RSA (indicating poorer vagal control over sympathetic reactivity) compared to TD controls in response to videos of people reading a story. In the same vein, Neuhaus and colleagues reported reduced RSA, increased HR, and decreased pre-ejection period (PEP; indicating increased sympathetic activity) in children with ASD vs. TD controls during a social interaction paradigm, albeit only when interacting with a familiar partner (Neuhaus, Bernier, & Beauchaine, 2016), unlike in van Hecke et al (2009) (see Table 2.2). In line with the theory that children with ASD display heightened

arousal during social engagement, several studies reported increased salivary cortisol levels in response to playground interaction paradigms in children with ASD relative to TD controls (Corbett, Schupp, & Lanni, 2012; Corbett, Schupp, Simon, Ryan, & Mendoza, 2010; Schupp, Simon, & Corbett, 2013) (see Table 2.3). In addition, Naber and colleagues reported that toddlers with ASD had an increased cortisol response to caregiver separation in a Strange Situation paradigm (Naber et al., 2006). However, the evidence was not always consistent. Some studies found no difference between children with ASD and TD controls in RSA or HR response to social challenge, i.e. a conversation with the examiner (Klusek, Martin, & Losh, 2013) or the stranger approach paradigm (Sheinkopf, Neal-Beevers, Levine, Miller-Loncar, & Lester, 2013).

Notably, findings from social engagement paradigms appear inconsistent with studies that used psychosocial stress tests, such as the TSST, in youth with ASD. In contrast to social engagement paradigms, studies using psychosocial stress tests reported decreased sympathetic reactivity (Panju, Brian, Dupuis, Anagnostou, & Kushki, 2015), decreased heart rate, and no difference in parasympathetic activity in response to the challenge in children with ASD compared to TD controls (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014; Jansen, Gispen-de Wied, van der Gaag, & van Engeland, 2003; Kushki, Brian, Dupuis, & Anagnostou, 2014), although evidence is not always consistent (Klusek et al., 2013; Levine et al., 2012) (see Table 2.2). Similarly discrepant evidence can be found for HPA axis responsiveness to social stressors. In contrast to social engagement paradigms where children with ASD showed higher cortisol reactivity compared to TD controls, TSST studies reported relatively dampened cortisol responses to stress in ASD youth (Corbett et al., 2012; Hollocks et al., 2014; Lanni, Schupp, Simon, & Corbett, 2012; Levine et al., 2012) (see Table 2.3). One study reported no difference in cortisol responsiveness to a public speaking task (Jansen et al., 2003); however the task used was markedly different from the original TSST in that the mental arithmetic task was removed and participants gave their speech to a one-way mirror instead of a panel of examiners in the room.

Table 2.2. Studies that compared ANS responses to psychosocial stress in youth with ASD and TD controls.

Citation	ASD n	TD n	ASD age	TD age	Stress paradigm	Main results in ASD group (vs. TD)	Comorbidities assessed?
Hollocks et al (2014)	52	23	12.8 ± 2.0	13.9 ± 1.9	TSST	No difference in HRV response; ↓ HR response	Yes; lowest HR response in the ASD+anx group & HR responsiveness correlated with anxiety severity in ASD+anx
Jansen et al (2003)	10	12	9.4 ± 1.4	9.4 ± 1.5	Psychosocial stress test (public speaking)	↓ HR response relative to TD, but still a significant stressor-related increase. No HR increase during talk anticipation.	Yes; No difference in CBCL subscale scores between the groups; CBCL not correlated with HR.
Klusek et al (2013)	40	28	10.1 ± 3.0	8.8 ± 2.4	10 min conversation with an examiner	No difference in HR or RSA response	Yes; no association between CBCL anxiety and physiological responsiveness
Kushki et al (2014)	40	43	12 ± 2.9	12.5 ± 2.9	Public speaking task	↓ HR response; no difference in RSA response	Yes; HR reactivity was negatively correlated with GAD symptoms
Levine et al (2012)	19	11	9.7 ± 1.4	9.6 ± 1.4	TSST	No difference in skin conductance response (sympathetic) or vagal tone (parasympathetic)	No (exclusion criterion)
Neuhaus et al (2016)	18	18	10.0 ± 1.1	10.0 ± 0.9	Social interaction with novel vs. familiar social partners	↓ RSA overall. In response to novel partner: no difference; Familiar partner: ↑ HR, ↓ PEP (↑ sympathetic influence), ↓ RSA (↓ parasympathetic influence)	No
Panju et al (2015)	47	37	12.1 ± 2.9	12.5 ± 2.9	Public speaking task	↓ skin conductance (sympathetic)	Yes; ↓ skin conductance (sympathetic) in ASD+anx compared to both TD and ASD-anx
Sheinkopf et al (2013)	15	8	4.3 ± 1.2	3.6 ± 1.0	Social challenge: Stranger approach paradigm (proximal vs. distal)	No difference in HR or RSA response	No
Van Hecke et al (2009)	18	16	10.0 ± 1.6	9.9 ± 1.6	Watching videos of familiar vs. unfamiliar people	↓ RSA overall and ↓ RSA in response to unfamiliar people (greater parasympathetic withdrawal)	No

↓, reduced. ↑, increased. ASD+anx, participants with ASD and anxiety. ASD-anx, participants with ASD without anxiety. CBCL, Child Behavior Checklist. GAD, generalised anxiety disorder. HR, heart rate. HRV, heart rate variability. PEP, pre-ejection period. RSA, respiratory sinus arrhythmia. TD, typically developing. TSST, Trier Social Stress Test

Table 2.3. Studies that compared cortisol responses to psychosocial stress in youth with ASD and TD controls.

Citation	ASD n	TD n	ASD age	TD age	Stress paradigm	Response to stress in ASD group vs. TD	Comorbidities assessed?
Corbett et al (2010)	21	24	10.0 ± 1.1	9.9 ± 1.5	Social engagement (playground paradigm)	↑ salivary cortisol response	No
Corbett et al (2012)	27	32	10.1 ± 1.3	9.9 ± 1.6	TSST	↓ salivary cortisol response	No
Corbett et al (2012)	27	32	10.1 ± 1.3	9.9 ± 1.6	Social engagement (playground paradigm)	↑ salivary cortisol response	No
Hollocks et al (2014)	52	23	12.8 ± 2.0	13.9 ± 1.9	TSST	↓ salivary cortisol response	Yes; lowest cortisol response in the ASD+anx group & cortisol responsiveness correlated with anxiety severity in ASD+anx
Jansen et al (2003)	10	12	9.4 ± 1.4	9.4 ± 1.5	Public speaking task	No difference in salivary cortisol response	No difference in CBCL subscale scores between the groups; CBCL not correlated with cortisol.
Lanni et al (2012)	15	15	9.8 ± 1.3	9.6 ± 1.7	TSST	↓ salivary cortisol response	Yes; anxiety not related to cortisol response
Levine et al (2012)	19	11	9.7 ± 1.4	9.6 ± 1.4	TSST	↓ salivary cortisol response	No (exclusion criterion)
Naber et al (2006)	26	18	2.5 ± 0.5	2.4 ± 0.2	Strange Situation procedure (caregiver separation)	↑ salivary cortisol response	No
Schupp et al (2013)	26	26	10.2 ± 1.2	10.0 ± 1.5	Social engagement (playground paradigm)	↑ salivary cortisol response, especially in older children	No

↓, reduced. ↑, increased. ASD+anx, participants with ASD and anxiety. CBCL, Child Behavior Checklist. TD, typically developing. TSST, Trier Social Stress Test.

2.3.4.2 Possible sources of inconsistencies between social engagement and psychosocial stress test paradigms

Studies in the TD population, reviewed above, consistently report that participants display physiological arousal in response to the psychosocial stress tests due to the uncontrollable and social-evaluative nature of these paradigms (Dickerson & Kemeny, 2004; Gunnar et al., 2009). One reason for the comparably lower stress responsiveness in youth with ASD may be that children with ASD do not find the social-evaluative aspect of the challenge stressful. Only a handful of studies collected self-reported measures of task-related stress or anxiety following the TSST; however, of the studies that did collect such measures, none has reported differences in subjective stress between children with ASD and TD children (Hollocks et al., 2014; Jansen et al., 2003; Lanni et al., 2012). This suggests that both groups found the psychosocial paradigm equally stressful, although further research into the association between self-reported stress and physiological stress responsiveness in ASD is needed.

Based on the high level of comorbidity in youth with ASD (see Section 1.2) and different profiles of physiological stress responsiveness in internalising vs. externalising disorders (Section 2.3.3.2), it seems plausible to suggest that the relative influence of anxiety and irritability may at least in part explain the discrepancies in social stress responsiveness among youth with ASD. This seems especially likely in light of the fact that psychosocial tests like the TSST were shown to induce feelings of anger and irritability as well as anxiety in the TD population (Lupis et al., 2014; Moons et al., 2010). Indeed, evidence suggests that anxiety and irritability are tightly linked in ASD. One view is that symptoms of irritability may exacerbate when a person with ASD becomes anxious (Tantam, 2003). Consistent with this view, White et al (2009) noted that anxiety in children with ASD often manifests in externalising behaviours. Furthermore, irritability symptoms (severe mood problems) are more common in young people with ASD who are prone to experiencing affective states (Simonoff et al., 2012). It has also been proposed that stress and anxiety exacerbate challenging behaviours in people with ASD (Groden et al., 1994). However, a recent study reported an inverse relationship between social anxiety and verbal and physical aggression in adults with ASD traits (S. W. White, Kreiser, Pugliese, & Scarpa, 2012), once again highlighting that labels of irritability and aggression need not be used interchangeably (see Section 1.2.2.2). An alternative hypothesis is that irritability and anxiety are separate, yet intercorrelated constructs, consistent with findings in the TD population (Stringaris & Goodman, 2009b).

Despite the associations reported above, irritability and anxiety have not yet been investigated together in the context of physiological stress responsiveness among young people with ASD. Three studies examined the effects of anxiety on ANS responsiveness during tasks involving public speaking (Table 2.2). Two have reported that co-occurring anxiety led to a dampened HR response to stress (Hollocks et al., 2014; Kushki et al., 2014), and one noted lower

sympathetic reactivity indexed by skin conductance response (Panju et al., 2015). The study by Hollocks et al (2014) also measured cortisol response, and used a thorough, parent-reported diagnostic interview (CAPA) to ascertain co-occurring anxiety in the sample. The authors found that children with ASD who met DSM-IV criteria for an anxiety disorder (ASD+anx, n=32) had a significantly blunted cortisol response to the TSST relative to those without co-occurring anxiety (ASD-anx, n=20) and TD controls (n=23). In addition, dampened HR and cortisol responsiveness to stress was positively correlated with anxiety severity in the ASD+anx group (Hollocks et al., 2014). Reduced physiological stress responsiveness in those with anxiety appears contradictory to findings from the TD population, where anxiety was usually associated with increased physiological responsiveness to stress (Brand et al., 2011; van West et al., 2008). It is possible that co-occurring irritability symptoms may explain these apparently contradictory findings.

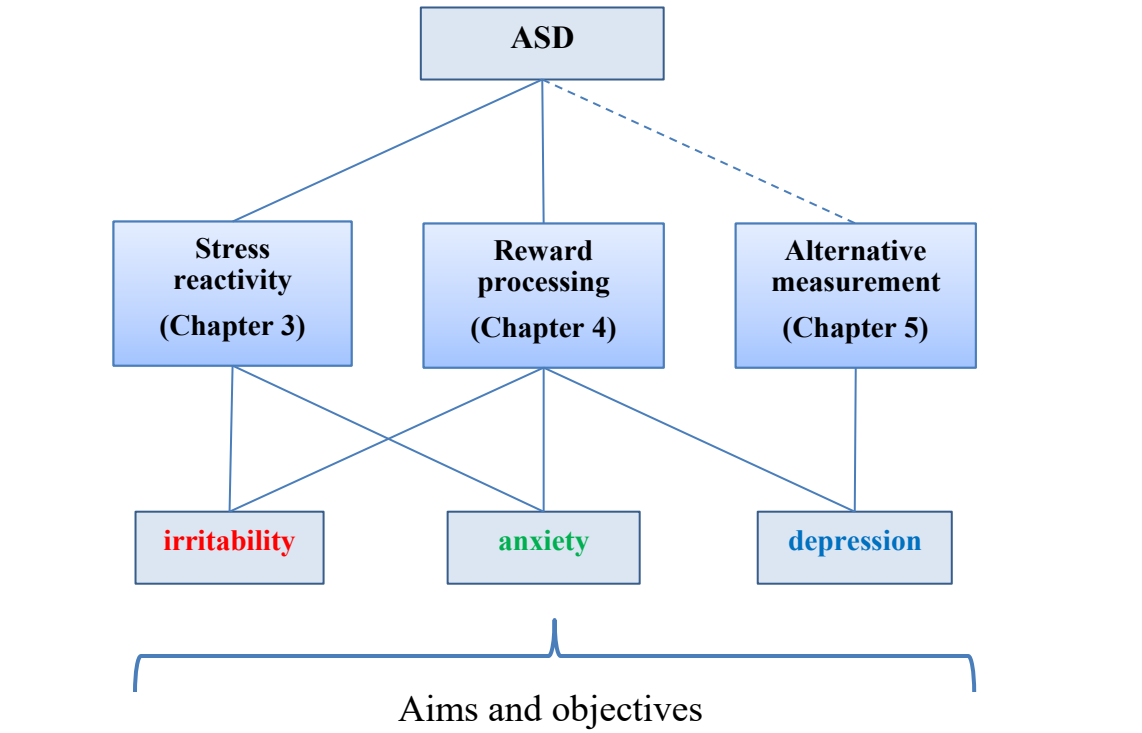
2.4 Interim summary

In this chapter I described the neurophysiological mechanisms of reward processing and stress responsiveness in the TD and ASD populations. The review of existing literature suggests that young people with ASD tend to respond to reward tasks and stress induction paradigms differently to TD youth, although the evidence is not clear-cut. Psychosocial stress paradigms appear to be the most effective in inducing ANS and HPA axis responsiveness in the TD population, while the corresponding stress response is often blunted in youth with ASD. Similarly, young people with ASD tend to show relatively reduced reward responsiveness, although reward sensitivity may differ depending on the stimuli used. Notably, studies in the TD population suggest that both reward processing and stress responsiveness differ as a function of co-occurring psychiatric symptoms, in particular anxiety and irritability. Yet, the influence of anxiety and irritability on reward processing and physiological stress responsiveness has not yet been sufficiently examined in youth with ASD. Understanding the relative influence of anxiety, irritability, and ASD symptoms in these processes may bring important insights about the mechanisms underlying the comorbidity between ASD and affective disorders.

2.5 Aims and objectives

In the two introductory chapters of this thesis I have reviewed the current state of evidence regarding mood and anxiety problems in young people with ASD. While it is clear that a large proportion of ASD youth also suffer from mood and anxiety problems, the research into the mechanisms underlying this overlap has been lagging behind that focusing on prevalence. Additional issues pertaining to comorbidity research in ASD youth include the measurement of mood and anxiety symptoms, and the fact that symptoms of anxiety, irritability, and depression often co-occur and/or correlate with each other. Figure 2.2 on the following page summarises these matters schematically and highlights the gaps in existing literature that this thesis will aim to address. Below I present specific aims and hypotheses of individual thesis chapters that relate directly to Figure 2.2.

Figure 2.2. Rationale for experimental studies presented in this thesis based on the literature reviewed in the introduction. Diagram shows (inter-correlated) symptoms that are often comorbid with ASD and possible explanations for the overlap. A brief description of the current state of evidence and relevant gaps in the literature are provided, along with aims and objectives – see main text for details. Different colours indicate existing evidence and research questions relevant to particular symptoms comorbid with ASD: irritability, anxiety, or depression. Dashed line indicates that study in Chapter 5 used healthy control subjects.



ANS, autonomic nervous system. *ASD*, autism spectrum disorder. *ASL*, arterial spin labelling. *HPA*, hypothalamic-pituitary-adrenal. *TD*, typically developing.

Chapter 3: Irritability in boys with ASD: measurement and associations with physiological stress reactivity

Measurement of irritability in ASD.

As discussed in Section 1.2.2.2, irritability has not been extensively studied in youth with ASD and most existing research has relied on parent- or teacher-reports of these problems. There is a need for scales that also incorporate the views of people with ASD themselves.

Aim: We examined symptom reporting of irritability as measured by the ARI (Stringaris, Goodman, et al., 2012) in 47 boys with high-functioning ASD, 40 boys with SMD, and 30 healthy control boys. Self- and parent-reported measures of irritability were obtained. We assessed internal consistency of the ARI and compared its item distribution patterns across the groups. We also examined parent-child reporting correspondence.

Hypotheses: We expected a moderate agreement between child- and parent-reported of irritability in the ASD sample, based on similar agreement estimates reported in a recent meta-analysis (Stratis & Lecavalier, 2013). We expected the pattern of irritability reporting to be similar between ASD and SMD groups but not healthy controls, based on the high level of irritability that youth with ASD experience (Simonoff et al., 2012) and the fact that the ARI differentiates well between children with SMD and healthy controls (Stringaris, Goodman, et al., 2012). However, we expected some inconsistencies in symptom reporting between boys with SMD and ASD, based on introspection difficulties that may limit the reliability of self-report in youth with ASD.

Irritability and physiological responsiveness to stress.

Although little research has been conducted on the mechanisms of irritability in young people with ASD, some have suggested that irritability may relate to anxiety and be associated with how individuals with ASD respond to stress (Groden et al., 1994). This hypothesis has not yet been studied experimentally.

Aim: We examined physiological responsiveness to a psychosocial stress test in 47 boys with ASD and 23 TD controls. Changes in salivary cortisol, HR and HRV throughout the test were recorded; and self- and parent-reports of irritability and anxiety were obtained. We used an existing dataset where the association between anxiety and physiological stress responsiveness was previously investigated (Hollocks et al., 2014) to examine the relative effects of irritability and anxiety on stress responsiveness.

Hypotheses: Based on previous studies that examined physiological reactivity with psychosocial stress tests, we expected boys with ASD to show a relatively reduced HR and cortisol response to the stress test (Corbett et al., 2012; Hollocks et al., 2014; Jansen et al., 2003; Kushki et al., 2014; Lanni et al., 2012; Levine et al., 2012). In addition, based on reduced

physiological stress responsiveness in TD youth with externalising symptoms (Hartman et al., 2013; van Goozen et al., 1998), we expected that reduced stress responsiveness in boys with ASD would be driven by high levels of irritability. We also explored whether co-occurring anxiety would limit the effects of irritability on stress-responsiveness, as reported in TD youth (van Goozen et al., 1998).

Chapter 4: Disentangling the autism-anxiety overlap: fMRI of reward processing in a community-based longitudinal study

Aberrations in the processing of reward have been reported in young people with ASD as well as in those with anxiety, irritability, and depression (see Figure 2.2 and Sections 2.2.3 and 2.2.4). However, no previous study has examined reward processing in ASD youth in relation to common comorbidities.

Aim: In this study, we investigated the interplay of ASD traits and anxiety during reward processing in a community sample of 1472 adolescents who performed a modified MID reward task. We tested the independent influence of each ASD traits and anxiety on brain correlates of reward processing; and also examined possible interaction effects between ASD traits and anxiety to assess whether combined ASD traits and anxiety is associated with distinct etiological mechanisms (Banaschewski et al., 2007). Additionally, we used a longitudinal design to assess whether neural responses during reward processing predict anxiety at two-year follow-up. Symptoms of depression and ODD were explored as potential confounders.

Hypotheses: The effect of comorbidities on neural correlates of reward processing in youth with ASD has not been previously investigated; hence the study was largely exploratory. Based on TD literature, we expected anxiety to be associated with increased activation in the ventral striatum and insula during reward anticipation (Bar-Haim et al., 2009; Guyer et al., 2006) and increased medial frontal activation following negative feedback (Helfinstein et al., 2011). However, we also expected that high levels of ASD traits may reduce this effect based on reduced reward sensitivity in youth with ASD (Chantiluke et al., 2015; Damiano et al., 2015).

Chapter 5: Measurement of mood states in young people: Exploring the feasibility of ASL and pattern recognition

As illustrated in Figure 2.2, one challenge pertaining to the research on mood and anxiety problems in ASD is the measurement of its symptoms, reflected in varying prevalence rates of various comorbidities, especially depression (see Section 1.2.3). There is a need for measures that would by-pass the limitations of self-reporting difficulties without relying on parent or observer ratings of these problems.

Aim: As a first step toward developing such measure of mood states in ASD, we examined whether arterial spin labelling (ASL), a neuroimaging technique especially suited for exploring longer-lasting states (see Section 5.2.1), is sensitive to experimentally induced mood changes in 21 healthy young people. We then employed pattern recognition techniques to explore whether sad, happy, and neutral mood states can be differentiated based on brain activation patterns alone, blind to the experimental mood condition. We used a healthy control sample rather than participants with ASD since the question was strictly methodological at this early development stage, reflected in the chapter's title.

Hypotheses: We hypothesised that the mood induction procedure will lead to significant changes in self-reported mood, and that it will generate brain activation changes in areas implicated in mood processing. Specifically, we expected that the subgenual anterior cingulate cortex would show higher activation following sad vs. neutral mood induction, based on previous mood induction studies in adults (Keightley et al., 2003; Liotti, Mayberg, McGinnis, Brannan, & Jerabek, 2002; Mayberg et al., 1999) and this region's hyperactivity in young people with depression reported in a recent ASL study (Ho et al., 2013). We also hypothesised increased rCBF in the ventral striatum following happy vs. neutral mood induction, based on a previous finding in healthy adults (Mitterschiffthaler, Fu, Dalton, Andrew, & Williams, 2007) and the relation between ventral striatal activity and euphoria in healthy adults (Drevets et al., 2001).

Overall, this thesis aims to address the gaps and/or inconsistencies in existing literature concerning the measurement and neurophysiological mechanisms of mood and anxiety problems in young people with ASD. Where possible, we consider anxiety, irritability, and depression together, based on the fact that these symptoms often co-occur or correlate with one another. Development of sensitive measurement tools and research into the underlying mechanisms are both important clinically for the appropriate identification and treatment of comorbid mood and anxiety problems in ASD youth.

Chapter 3 – Irritability in boys with ASD: measurement and associations with physiological stress reactivity

3.1 Abstract

Irritability in people with autism spectrum disorders (ASD) is common and impairing, yet its mechanisms remain understudied. We investigated symptom reporting and mechanisms of irritability in ASD, focusing on the relation between irritability and physiological stress responses. Forty-seven unmedicated boys with high-functioning ASD (hfASD) and 23 typically-developing boys aged 10-16 years completed a psychosocial stress test. Changes in cortisol, heart rate and heart rate variability throughout the test were recorded. Self- and parent-reported measures of irritability were obtained. Irritability symptom reporting in the hfASD group was compared to two groups of boys without ASD: highly-irritable boys (severe mood dysregulation, SMD; n=40) and healthy control boys (HC; n=30). Boys with hfASD scored significantly higher on irritability than HC boys, and they reported a pattern of irritability symptoms closely resembling that of boys with SMD. The internal consistency of irritability in hfASD was high by parent- and self-report. Although boys with hfASD showed significant stress-induced changes in cortisol and heart rate, those who rated themselves as highly-irritable had lower cortisol levels throughout the test compared to those low on irritability. Participants rated as highly-irritable by their parents showed blunted cortisol and heart rate responses to stress. The effects of irritability on heart rate, but not cortisol, were accounted for by trait anxiety. Our results suggest that irritability can be measured reliably in hfASD and is associated with distinct biological responses to stress.

3.2 Introduction

Autism spectrum disorder (ASD) is characterised by deficits in social reciprocity and communication, and by restricted, repetitive behaviours (APA, 2013). Additionally, children with ASD often display high levels of irritability (Mandy et al., 2014; Mayes et al., 2015; Simonoff et al., 2012). However, little research has been conducted on the mechanisms of irritability in children with ASD. This is surprising because, as noted in Section 1.2.2.1, irritability in typically developing (TD) children is associated with long-term adverse outcomes (Copeland et al., 2014; Dougherty et al., 2013; Leibenluft, 2011; Mikita & Stringaris, 2013; Stringaris et al., 2009). Here, we use a multi-method, multi-informant experimental approach to investigate irritability in boys with high-functioning ASD (hfASD).

In previous studies of children with ASD, the term 'irritability' was often used to describe severe behavioural difficulties, e.g., verbal and physical aggression, self-injury or property destruction. Such behaviours feature, for example, on the irritability subscale of the ABC (Aman et al., 1985). By contrast, in TD children, irritability refers to a mood that may or may not lead to aggression (Leibenluft, 2011; Mikita & Stringaris, 2013; Stringaris, Goodman, et al., 2012). Despite these purported differences, recent studies suggest that irritability in ASD and TD youth may share important characteristics. For instance, irritability in TD children has stronger phenotypic and genetic associations with depression than with delinquency (Stringaris, Zavos, et al., 2012). Likewise, in a cross-sectional study of children with ASD, Mandy et al. (2014) identified that while DSM-5-defined argumentative and defiant behaviour was associated with externalising problems, angry/irritable symptoms predicted internalising problems. It is important to distinguish between irritable mood and acts of hostility or aggression, as their mechanisms may be different (Stringaris, 2011). An additional problem with scales such as the ABC is that they were originally developed for people with intellectual disability and include symptoms, such as screaming, that are less common in hfASD (Arnold et al., 2003). Moreover, there is a need for scales that are not just observer- or parent-rated but also incorporate the views of people with ASD themselves.

In TD children, chronic irritability was studied extensively under the term severe mood dysregulation (SMD), characterised by frequent temper outbursts with irritability between outbursts (Leibenluft et al., 2003). Childhood SMD predicts depression in adolescence (Brotman et al., 2006), consistent with the well-established longitudinal association between irritability and internalising problems (Krieger et al., 2013; Mandy et al., 2014; Stringaris & Goodman, 2009a, 2009b). Recently, Simonoff et al. (2012) demonstrated that parent-reported mood dysregulation symptoms identified adolescents with ASD who had higher rates of comorbidity. This suggested that mood dysregulation in young people with ASD might resemble that of TD children. However, mood dysregulation was not limited to irritability but broadly defined using questions about sad mood, mood lability and explosive rage and did not include data on self-reported irritability.

The present study addresses the gaps in existing literature by focusing on two sets of questions. The first concerns recognition and measurement of irritability in young people with hfASD. Arguably, children with hfASD may underreport their irritability symptoms due to introspection difficulties, as proposed by Mazefsky, Kao and Oswald (2011) who found low correspondence between a parental diagnostic interview and self-reports of depression, anxiety and ADHD in youth with hfASD. Here we measure irritability using the self- and parent-reported Affective Reactivity Index (ARI) (Stringaris, Goodman, et al., 2012), which was previously shown to be reliable in TD children (DeSousa et al., 2013; Mulraney, Melvin, & Tonge, 2014; Stringaris, Goodman, et al., 2012) and distinguished between children with SMD and healthy controls (Stringaris, Goodman, et al., 2012). We assess consistency of reporting and compare the symptom pattern of irritability in boys with hfASD to that of boys with severe irritability (SMD) and healthy controls. We also investigate whether the strong cross-informant agreement for irritability symptoms in TD children (Stringaris, Goodman, et al., 2012) is present in our hfASD sample.

The second set of questions concerns mechanisms underlying irritability in ASD. We test the hypothesis that irritability may be associated with how individuals with ASD respond to stress. As described in more detail in Section 2.3.1, physiological mechanisms of stress-response include the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. Under threat, activation of the sympathetic branch of the ANS prepares an individual to deal with the stressor, resulting in heightened arousal, e.g., increased heart rate (HR). The body's return back to homeostasis is controlled by the parasympathetic branch of the ANS. The HPA axis, which encompasses a cascade of biochemical reactions, is also activated following stress exposure, resulting in increased levels of cortisol that peak around 20 minutes post-stressor (Romanczyk & Gillis, 2006). Evidence from TD adult studies suggests that fear and anger responses to stress may have different underlying physiological profiles. For example, Moons et al. (2010) distinguished between self-reported anger and fear responses to stress, which differentially influenced the HPA axis. Anger-driven, confrontational stress responses were associated with greater stress-induced increase in cortisol, while fear reactions were associated with a decrease in cortisol levels. This suggests that a tendency to respond to stress in an irritable manner may be associated with a distinct pattern of physiological activation. However, self-reported anger and fear were moderately-correlated in the Moons et al. (2010) study, making disentanglement of the two emotions difficult, and evidence in the opposite direction was also reported (Herrero et al., 2010; Kazen et al., 2012) (see Section 2.3.3).

Children with ASD often show reduced physiological responsiveness to psychosocial stress compared to the TD population. While stress paradigms such as the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993) reliably induce elevated cortisol levels in the TD population (Dickerson & Kemeny, 2004; Gunnar et al., 2009), children with ASD often show a blunted cortisol response to psychosocial stressors (Lanni et al., 2012; Levine et al., 2012), although these

results were not always replicated (Jansen et al., 2003). Research into ANS responses to stress, e.g. HR or skin conductance changes, also produced mixed results. While some studies found children with ASD to respond differently to stressors relative to controls (Goodwin et al., 2006; Hollocks et al., 2014; Jansen et al., 2003; Kushki et al., 2014), others found no such differences in ANS stress responsiveness (Klusek, Roberts, & Losh, 2015; Levine et al., 2012).

This inconsistency may be partly explained by the relative contributions of irritability and anxiety in stress response. Research suggests that youth with ASD show greater levels of anxiety than those in community populations, and that anxiety levels of children with ASD are comparable to those of clinically anxious children (for a review see MacNeil, Lopes, & Minnes, 2009). Furthermore, mood dysregulation is more common in adolescents with ASD who display symptoms of anxiety (Simonoff et al., 2012) and it was suggested that irritability may worsen when a person with ASD becomes anxious (Groden et al., 1994; Tantam, 2003). Additionally, irritability is common and impairing among TD children with anxiety disorders (Krebs et al., 2013; Stoddard et al., 2014). It is therefore important to examine how irritability is related to stress responses in children with ASD and how this relation is influenced by co-occurring anxiety. As mentioned above, the two emotions may be difficult to distinguish.

We use a multi-method, multi-informant experimental approach to investigate irritability, anxiety, and physiological reactivity to stress in boys with hfASD. We first investigate whether irritability can be measured reliably in boys with hfASD using a concise scale. Second, we examine irritability and anxiety in boys with hfASD in relation to their HR, HR variability, and cortisol levels following a psychosocial stress test. Based on extant literature, we expected boys with hfASD to show a relatively reduced HR and cortisol response to the stress test (Corbett et al., 2012; Hollocks et al., 2014; Jansen et al., 2003; Kushki et al., 2014; Lanni et al., 2012; Levine et al., 2012). In addition, based on reduced physiological stress responsiveness in TD youth with externalising symptoms (Hartman et al., 2013; van Goozen et al., 1998), we expected that reduced stress responsiveness in boys with hfASD would be driven by high levels of irritability. We also explored whether co-occurring anxiety would limit the effects of irritability on stress-responsiveness in boys with hfASD, as found in TD young people (van Goozen et al., 1998).

3.3 Methods

3.3.1 Sample

3.3.1.1 Participants with hfASD

Fifty-four male participants with hfASD aged 10-16 were recruited from clinics in London and the South-East of the UK. All participants had a full-scale IQ≥70 on the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) and were not taking psychotropic medications. ASD diagnoses were made by expert clinicians and, in 31/54 cases, confirmed using either the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994) or the Autism Diagnostic Observation Schedule-Generic (ADOS-G) (Lord et al., 2000). In absence of ADI/ADOS confirmed diagnosis, a Social Communication Questionnaire (SCQ) (Rutter et al., 2003) score of ≥15 and clinical diagnosis were required. Two participants were excluded based on this criterion. Of the remaining sample, we obtained irritability measurements from 47 participants.

3.3.1.2 Non-ASD participants

Two independent control samples of boys without ASD were used, each to answer a different research question. First, to answer the question about measurement of irritability in ASD, we used 40 boys with SMD (age 12.6 ± 2.6, 8-17 years) and 30 healthy control (HC) boys (age 11.5 ± 3.6, 6-18 years) studied in our previous published work on youth irritability (Stringaris, Goodman, et al., 2012). This sample provided data on self- and parent-reported irritability (ARI) but did not complete the stress test. Second, to examine the role of irritability in shaping physiological responses to stress, we used an independent sample of 23 TD boys aged 10-16 who completed both the psychosocial stress test and ARI questionnaires. This sample was recruited from local London schools and through public advertisement, concurrently with our ASD sample, and had no parent-reported history of psychiatric or neurological problems.

The study of physiological stress responses was approved by the South East London Research Ethics Committee (10/H0870/67). Written informed consent was obtained from all participants.

3.3.2 Symptom assessment

Irritability was measured using the Affective Reactivity Index (ARI) (Stringaris, Goodman, et al., 2012), a 6-item scale that is both parent- and self-reported. The ARI asks about symptoms of irritability in the previous 6 months and includes a 7th item assessing impairment due to irritability.

The scale showed excellent internal consistencies in TD children, with Cronbach's alphas 0.89 (parent-report) and 0.90 (self-report) (Stringaris, Goodman, et al., 2012).

SMD was assessed using a supplementary module of the Schedule for Affective Disorders and Schizophrenia (K-SADS) (Kaufman et al., 1997; Leibenluft et al., 2003) and defined by persistent abnormal mood (anger or sadness), hyperarousal and increased reactivity to negative emotional stimuli (for full SMD criteria see Leibenluft et al., 2003). SMD precludes the diagnosis of ASD or bipolar disorder.

Trait anxiety was measured with the Spence Children's Anxiety Scale (SCAS) (Spence, 1998) parent and child versions, a 44-item screening questionnaire providing global measurement of most childhood anxiety symptoms.

3.3.3 Procedure

The Psychosocial Stress Test (PST) was carried out in the afternoon, beginning between 1300h and 1400h, to reduce the impact of diurnal cortisol variation. Participants were asked not to consume any food or drink within 30 minutes of task initiation. Participants were told they would undertake a mildly stressful task, preceded by 40 minutes of relaxation and followed by a 40-minute recovery period in which they would watch cartoons. The BioHarness HR telemetry system was then placed onto the participants.

The PST was a modified version of the TSST (Kirschbaum et al., 1993) with the mental arithmetic task replaced with the Rey-Osterrieth Complex Figure test (Osterrieth, 1944; Rey, 1941). This modification was based on our clinical observation that some of the hfASD boys may have found the mental arithmetic task less stressful than TD controls. During the 20-minute stress paradigm, participants were asked to: copy a complex figure, prepare a speech about themselves in 10 minutes, give a 5-minute presentation and remember and reproduce the complex figure under timed conditions. Up until the end of speech preparation, two researchers were present in the room. Then, a third person (unknown to the participant, presented as an evaluator) entered the room asking the participant to begin their presentation. Participants were required to stand for five minutes and standardised prompts were given after 30s of silence.

Salivary cortisol was collected six times throughout the PST: twice during rest (-40min, -20min), pre-stressor (0min), post-stressor (+20min) and twice during recovery (+40min, +60min). Saliva samples were collected in plain Sarstedt salivettes and stored at -40°C. Saliva cortisol concentrations were determined using 'Immulite' Siemens Immunoassay system (www.diagnostics.siemens.com) (Mondelli et al., 2010).

Heart Rate (HR) was recorded continuously throughout the PST. HR electrocardiogram (ECG) was measured at 250_{Hz} using the Zephyr BioHarness wireless telemetry system. The BioHarness is a small, lightweight device worn around the chest via an unobtrusive strap that records human physiology signals, including ECG, respiration rate and temperature. It was shown

to be reliable and valid (Johnstone, Ford, Hughes, Watson, & Garrett, 2012a, 2012b) in laboratory and field settings (Johnstone, Ford, Hughes, Watson, Mitchell, et al., 2012). The ECG signal was recorded and analysed with Labchart 7 (ADInstruments Pty Ltd, Bella Vista, Australia). Mean HR values were extracted and divided into five segments: first 20 minutes of rest; second 20 minutes of rest; 20 minutes of stress test; first 20 minutes of recovery and second 20 minutes of recovery, in order to mirror cortisol analyses.

HR variability (HRV) analysis was conducted using the Labchart HRV module. The ECG recording was segmented into nine 5-minute long segments selected across the 100 minutes of recording, as recommended by the Task Force of the European Society of Cardiology (1996). Three blocks were taken from the rest phase, two from speech preparation, one from speech and three from the recovery phase. The signal was pre-processed with a low-pass filter before R-wave to R-wave (RR) intervals were automatically identified. Detected RR intervals were then manually inspected for errors. Ectopic RR intervals were excluded and subsequently interpolated and replaced by the average of nearest preceding and succeeding normal – normal intervals (NN) within the block. We conducted a spectral HRV analysis using Fast Fourier Transform. HRV falling within the high-frequency domain (HF; .15-.40Hz) is considered a measure of vagal tone and lower values indicate lower parasympathetic modulation. To isolate the relative contribution of the sympathetic modulation, we calculated a ratio between the low-frequency domain (LF; .04-.15Hz; representing combined parasympathetic and sympathetic input) and the HF (Montano et al., 1994), as described previously in Hollocks et al (2014).

Subjective stress responses (rated on a scale from 1 to 10) were collected six times during the PST following salivary cortisol collection. In addition, after the PST concluded, participants answered a series of yes/no questions about their experience during the PST, indicating whether or not the stress test invoked particular emotions or states (see Table 3.2).

3.3.4 Data analysis

We analysed the data with SPSS 20 (IBMCorp, 2011) and Stata 11 (StataCorp, 2009). Cortisol was non-normally distributed and therefore was *l*og-transformed. We collected a total of 44 parent-reported and 29 self-reported ARI questionnaires for participants with hfASD (26 had both self- and parent-reported ARI, 3 only had self- and 18 only had parent-reported ARI). For TD controls, we collected 20 parent-reported and 9 self-reported questionnaires (9 had ARI data from both informants). There were no differences in IQ between participants with hfASD who completed the ARI and those who did not. Missing data for other key variables were limited (see Table 1). Age effects on all variables were examined using one-way analyses of variance (ANOVAs) with age as a continuous variable. Age had an effect on cortisol levels during the pre-stress rest period in the hfASD group (p=.002) and was therefore added as a covariate in hfASD cortisol analyses.

Measurement of irritability in hfASD. Cronbach's alpha coefficients assessed internal consistency of the ARI in boys with hfASD vs. controls. Pearson correlation coefficients estimated the parent-child reporting correspondence. We then compared the item distribution pattern of the ARI in boys with hfASD to that of boys with SMD and HC boys. Finally, we examined how the severity of irritability symptoms related to impairment using ANOVA on ARI total score and ARI impairment item.

Irritability and anxiety. Pearson correlation coefficient estimated the relation between irritability and SCAS scores, separately for self- and parent-report.

Psychosocial Stress Test. Paired-samples t-tests and repeated-measures ANOVAs were performed to examine the efficacy of the PST in producing stress-induced changes to cortisol levels, HR, HRV, and subjective stress responses, separately for hfASD and TD controls. Chi square tests were used to examine whether participants with hfASD and TD controls differed in the frequency of specific emotions or states they experienced during the PST, as measured by the post-test questionnaire.

Irritability and physiological responses to stress in hfASD. We used two complimentary analysis strategies to examine the effects of irritability on physiological stress responsiveness in boys with hfASD.

Consistent with previous studies (Lanni et al., 2012; van Goozen et al., 1998) we conducted repeated-measures ANOVAs, with continuous irritability score entered as a covariate, separately for self- and parent-report. The effects of anxiety, on physiological stress-responsiveness were assessed by adding trait anxiety (parent- or self-reported SCAS) as a covariate. For illustration purposes, we used a median split (low vs. high ARI score) to depict the results in figures. Since we were primarily interested in physiological reactivity to the experimental stressor, only the time points that directly test our hypothesis were used in the analysis. For cortisol, these were immediately before (0min) and immediately after the stressor (+20min). For HR, three middle phases were used: second rest phase served as a baseline and was compared to the stress condition, while the first recovery period was used to evaluate return to baseline post-stressor.

ANOVA analyses were complemented by piecewise linear regression models. The response profiles of cortisol and HR (average values at all time points and slope decline/increase rates) exhibited non-linear relationships with time. Piecewise models can fit any type of non-linear relationships by assuming parametric relationships within smaller segments (Marsh & Cormier, 2001). Unlike ANOVAs, they allow formal hypothesis-testing about the mean and slope of the response profile within segments. We fitted piecewise linear mixed models to the non-linear profiles by dividing the time axis (all time points) into three segments using pre-specified knot points. Based on an exploratory analysis of the response profiles, we fitted three-piece linear mixed models using two knot points, one at just before the initiation of the psychosocial stress and the other corresponding to the peak/nadir of the stress response profile. For cortisol, the peak

of the stress response profile corresponded to the saliva sample obtained post-stressor (+20min). For HR, the peak of the stress response profile corresponded to HR readings averaged over the 20-min stress phase. This partitioning allows convenient modelling of the physiological parameter profiles by using different parameterization for the rest, stress and recovery periods and allowing hypothesis-testing to compare the parameters within periods. The analyses were performed separately for self- and parent-reported irritability. Participants were assigned into groups of low vs. high irritability using a median split on the total irritability score. We then tested the role of anxiety in shaping physiological stress response profiles, by fitting a separate piecewise regression model with anxiety (total SCAS score, self- or parent-reported) into the model as a covariate.

3.4 Results

3.4.1 Participant characteristics

Participant characteristics and data for key physiological variables are presented in Table 3.1.

Table 3.1. Means ± standard deviations (ranges) and sample sizes for key variables in the study in participants with high-functioning autism spectrum disorders (hfASD) and typically-developing (TD) controls.

	hfASD	n	TD controls	n
Participant Characteristics				
Age	12.8 ± 2.0 (10-16)	52	13.9 (1.9, 10-16)*	23
IQ	101.2 ± 13.5 (76-138)	52	117.7 ± 9.1 (96-136)***	23
SCQ	23.2 ± 6.4 (12-36)	50	1.5 ± 1.5 (0-6)***	23
Irritability				
parent-reported	7.6 ± 3.0 (0-12)	44	0.6 ± 0.8 (0-3)***	20
self-reported	5.1 ± 3.1 (0-12)	29	2.6 ± 1.7 (0-5)*	9
Anxiety				
parent-reported	33.9 ± 19.0 (3-88)	50	6.9 ± 5.1 (0-24)***	23
self-reported	31.1 ± 15.8 (3-72)	50	12.0 ± 6.4 (1-25)***	22
Psychosocial Stress Test				
Subjective stress rating				

before test	2.0 ± 1.9 (1-9)	46	1.6 ± 1.2 (1-6)	23
after test	5.2 ± 2.6 (1-10)	43	4.6 ± 2.2 (1-8)	22
***log* cortisol**				
before test	1.4 ± 0.4 (0.6-2.4)	52	1.4 ± 0.3 (0.7-2.0)	23
after test	1.5** (0.4, 0.5-2.2)	50	1.8** (0.5, 1.0-3.1)	22
Heart rate (bpm)				
before test	84.4 ± 11.1 (62.9-109.8)	51	76.5± 9.2 (60.2-93.6)**	23
during test	89.3 ± 11.2 (67.1-120.8)	50	87.0 ± 10.8 (66.6-103.9)	22
after test	80.9 ± 11.2 (57.4-105.3)	49	72.9 ± 9.1 (59.1-90.5)**	20
Heart rate variability: spectral analysis (NU)				
before test	53.4 ± 17.0 (24.1-96.3)	46	50.6 ± 15.7 (32.4-88.3)	21
during test	32.7 ± 12.9 (10.4-60.0)	49	30.6 ± 9.8 (12.6-48.3)	20
after test	53.8 ± 14.9 (26.2-83.9)	45	47.7 ± 16.9 (21.3-77.1)	20
Heart rate variability: LF/HF ratio analysis				
before test	1.7 ± 1.0 (0.5-4.3)	46	1.9 ± 0.9 (0.6-3.3)	21
during test	2.2 ± 1.7 (0.4-8.1)	49	2.3 ± 1.4 (0.8-6.0)	20
after test	1.7 ± 1.0 (0.3-4.1)	45	2.3 ± 1.4 (0.6-5.3)	20

* $p < .05$; ** $p < .01$; *** $p < .001$. LF/HF low frequency/high frequency.

3.4.2 Reliability and item profile of irritability in boys with hfASD

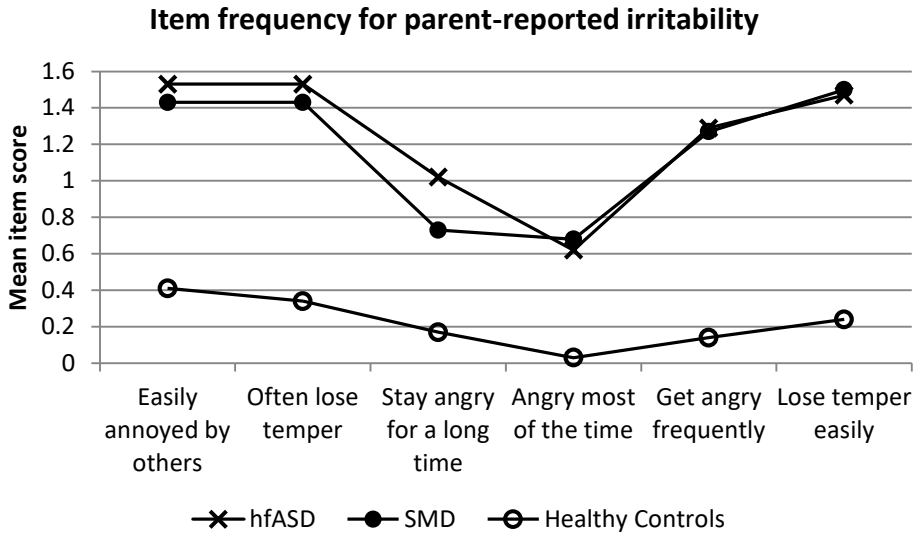
Internal consistency. Our first aim was to check whether the irritability scales were internally reliable in hfASD. The ARI showed excellent internal consistency with Cronbach's alphas 0.82 (parent report) and 0.80 (self report). This was compared to 0.91/0.86 in boys with SMD, 0.83/0.21 in HC boys (note: 15 self reports) and 0.43/0.31 in TD boys (note: 9 self reports).

Cross-informant agreement. Next, we checked whether irritability reported by boys with hfASD related to their parents' report of these problems. There was a moderately high correlation between parent- and self-reported scales, $r(26)=.55$, $p=.003$. Irritability scores were significantly higher for parent- than self-report in boys with hfASD, $t(25)=4.10$, $p<.001$, $d=0.76$ (Table 3.1). Similar differences between parent- and self-reported irritability were observed in boys with SMD (7.0 ± 3.3 vs. 4.7 ± 2.8, respectively) and HC boys (1.3 ± 2.0 vs. 0.3 ± 0.5, respectively). Cross-informant coefficients for SMD, HC and TD groups were 0.60, 0.43, and 0.64, respectively.

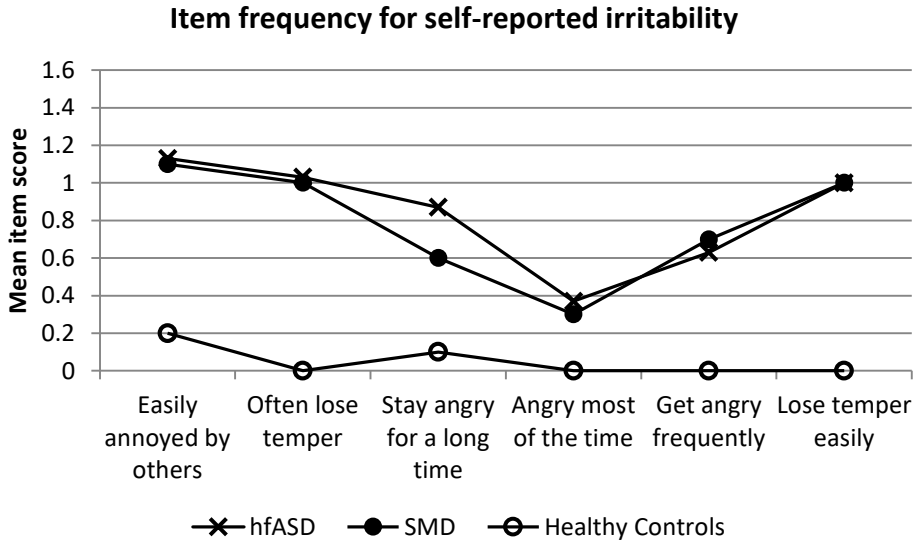
Item distribution. We then compared the symptom pattern of irritability in boys with hfASD to that of boys with SMD and HC boys. The pattern of irritability symptoms in hfASD closely matched to SMD (Figure 3.1 A and B). Being easily annoyed was the most common item, while the duration item 'angry most of the time' was reported least by both reporting sources. HC boys scored significantly lower than those with SMD or hfASD on all items of the ARI.

Figure 3.1. Item frequencies for parent- and self-reported irritability in boys with high-functioning autism spectrum disorders (hfASD) compared to boys with severe mood dysregulation (SMD) and healthy controls.

A.

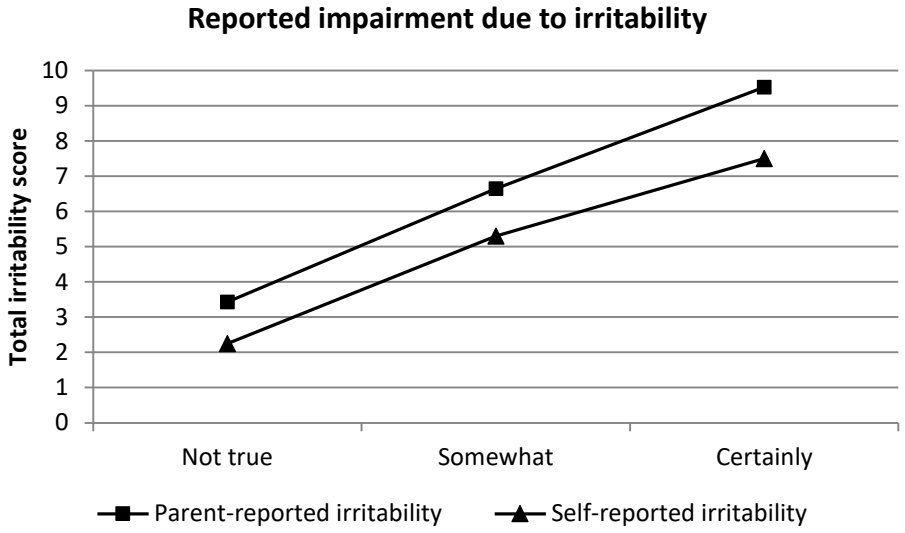


B.



Impairment. Our next aim was to investigate the extent to which boys with hfASD perceive their irritability as impairing. Indeed, as illustrated in Figure 3.2, increases in irritability symptoms were strongly associated with increases in reported impairment due to irritability, by either reporting source [parent-reported irritability: $F(2,41)=25.47$, $p< .001$; self-reported irritability: $F(2,25)=11.10$, $p<.001$].

Figure 3.2. The relation between impairment due to irritability and total irritability score in boys with high-functioning autism spectrum disorders (hfASD).



3.4.3 Irritability and anxiety

Parent reports of irritability and anxiety were strongly correlated, $r(44)=.49$, $p=.001$. In contrast, self-reported irritability did not correlate significantly with self-reported anxiety, $r(28)=.05$, $p=.791$. No significant correlations between irritability and anxiety were found in TD boys; $r(20)=.22$, $p=.353$ (parent report) and $r(9)=.40$, $p=.286$ (self report).

3.4.4 Psychosocial Stress Test

There was a significant and statistically equal [$F(1,63)=1.32$, $p=.256$] rise in subjective stress for boys with hfASD [$t(42)=8.13$, $p<.001$, $d=1.37$] and TD boys [$t(21)=6.14$, $p<.001$, $d=1.60$]. In addition, the groups did not differ in their self-reported stress experience during the PST (see Table 3.2).

Table 3.2. Comparison of self-reported stress experience between boys with hfASD and TD control boys (% "Yes" answers to the questions below following the psychosocial stress test).

During the test I…	% hfASD (n=31)	% TD controls (n=14)	Chi-Square Test
...felt annoyed	25.8	28.6	X^2=0.04, p= .846
...felt angry	9.7	0	X^2=1.45, p= .228
...lost my temper	6.5	0	X^2=0.95, p= .331
…felt anxious or scared	71.0	42.9	X^2=3.24, p= .072
…felt like crying	6.5	0	X^2=0.95, p= .331
…felt dizzy	19.4	14.3	X^2=0.71, p= .681
…felt sick	12.9	0	X^2=1.98, p= .159
…felt unable to think	67.7	64.3	X^2=0.05, p= .820

The psychosocial stressor significantly increased cortisol levels in boys with hfASD, $t(49)=2.10$, $p=.041$, $d=0.30$, and TD boys, $t(21)=3.64$, $p=.002$, $d=0.89$. The rise in cortisol levels was significantly steeper in TD boys, $F(1,70)=5.39$, $p=.023$, $\eta_p^2=.072$. The stress test also had an effect on the participants' HR, in both boys with hfASD, $F(2,92)=85.99$, $p<.001$, $\eta_p^2=.651$, and TD controls, $F(1.35,25.66)=74.30$, $p<.001$, $\eta_p^2=.796$. TD boys again showed a stronger physiological reactivity to the stressor, $F(1.65,108.91)=8.58$, $p=.001$, $\eta_p^2=.115$. HR variability changed significantly throughout the PST in boys with hfASD [spectral analysis: $F(2,78)=51.42$, $p<.001$, $\eta_p^2=.569$; LF/HF ratio analysis: $F(1.44,56.17)=4.44$, $p=.027$, $\eta_p^2=.102$]. TD boys displayed a change in HRV in the spectral [$F(2,38)=18.96$, $p<.001$, $\eta_p^2=.636$] but not ratio analysis [$F(2,38)=0.89$, $p=.419$]. However, HRV response profiles during the PST did not differ significantly between the groups [spectral analysis: $F(1,58)=1.53$, $p=.222$; LF/HF ratio analysis: $F(1,58)=1.15$, $p=.289$]. Overall, the PST was successful in producing self-reported and physiological changes.

3.4.5 Irritability and physiological responses to stress in hfASD: Repeated-measures ANOVAs

To explore the relation between irritability and stress-induced changes in cortisol levels, HR, and HRV in hfASD, we first conducted repeated-measures ANOVAs with irritability as a predictor. Age was added as a covariate in all cortisol analyses.

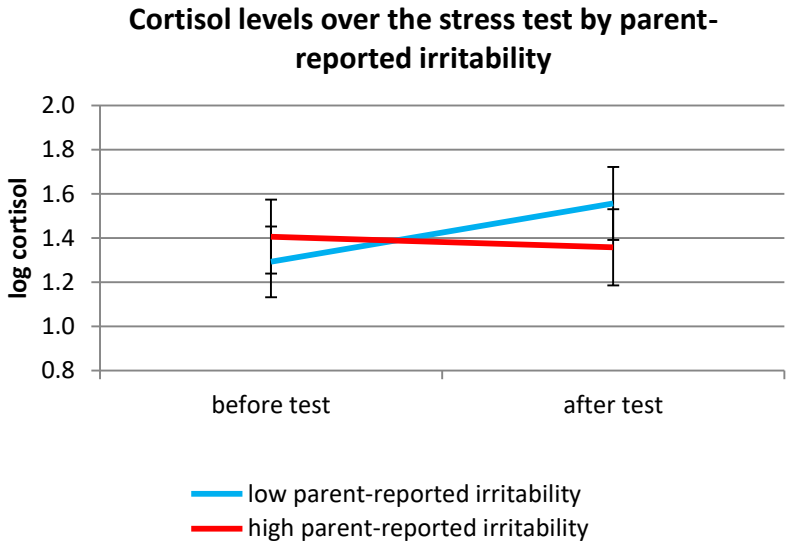
3.4.5.1 Cortisol reactivity

Parent report. We found a time-by-irritability interaction for parent report, $F(1,39)=8.71$, $p=.005$, $\eta_p^2=.182$. Figure 3.3 A illustrates this interaction schematically, using a median split to show that boys with high parent-reported irritability had a relatively dampened cortisol response to stress compared to those with low parent-reported irritability. We then examined whether anxiety affected the stress-induced change in cortisol levels. However, the time-by-irritability interaction effect remained significant after adding parent-reported anxiety into the model as a covariate, $F(1,38)=5.05$, $p=.031$, $\eta_p^2=.117$. No independent significant effects of parent-reported anxiety on cortisol levels were found when both irritability and anxiety were added into the model.

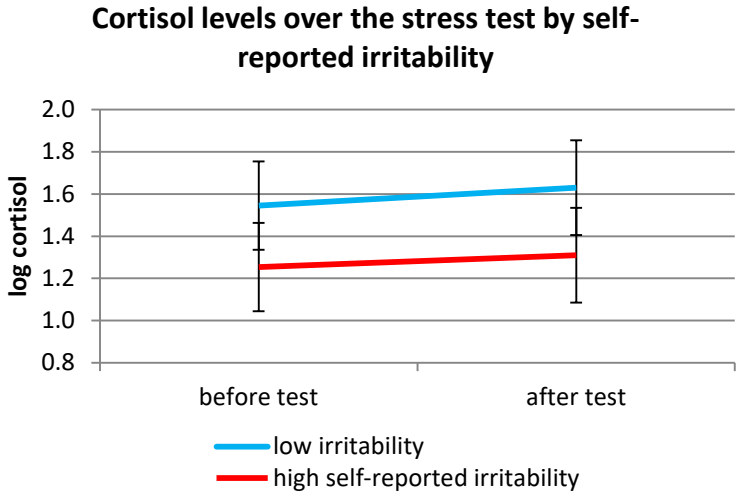
Self report. Irritability had a main effect on cortisol levels before and after the stressor, $F(1,25)=7.10$, $p=.013$, $\eta_p^2=.221$. Compared to adolescents with low self-reported irritability, those with high self-reported irritability showed lower cortisol levels irrespective of time (Figure 3.3 B). Similarly to parent report, the effect of self-reported irritability on cortisol levels remained significant after adding self-reported anxiety to the model as a covariate, $F(1,23)=4.96$, $p=.036$, $\eta_p^2=.177$. No significant effects of self-reported anxiety on cortisol levels were found when both irritability and anxiety were added into the model.

Figure 3.3. Pre- and post-stressor comparisons of cortisol levels for low vs. high parent- and self-reported irritability (median split) in boys with high-functioning autism spectrum disorders (hfASD) (95% confidence intervals).

A.



B.

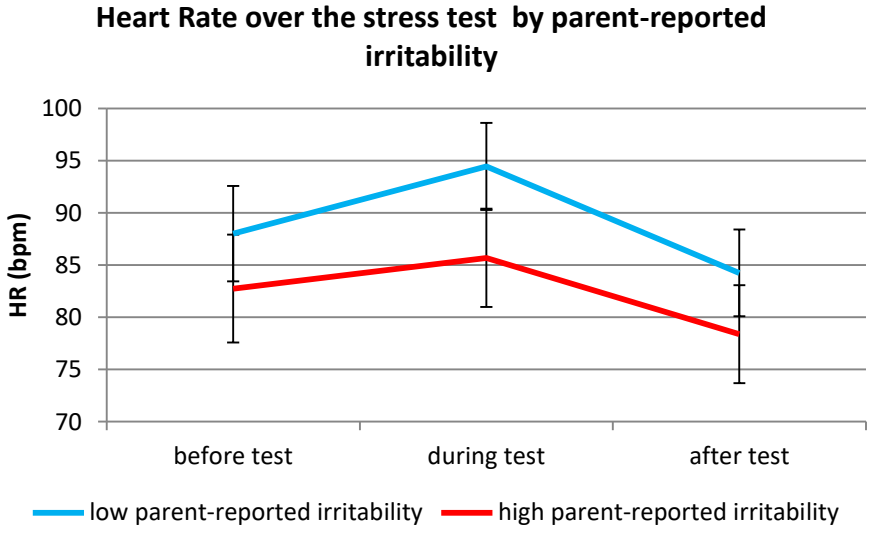


3.4.5.2 Heart rate

Parent report. We found a time-by-irritability interaction for parent report, $F(1.65,64.24)=6.10$, $p=.006$, $\eta_p^2=.135$. Boys with high parent-reported irritability displayed a dampened HR response to the stressor compared to boys with low parent-reported irritability (Figure 3.4). However, this effect was no longer significant after parent-reported anxiety was added into the model as a covariate. Instead, there was a significant interaction between parent-reported anxiety and time, $F(1.624,61.726)=4.012$, $p=.031$, $\eta_p^2=.095$. Boys with high parent-reported anxiety displayed a dampened HR reaction to the stressor compared with boys with low parent-reported anxiety.

Self report. No significant effects of self-reported irritability or anxiety on HR reactivity to stress were found.

Figure 3.4. The relation between parent-reported irritability (median split, low vs. high) and heart rate before, during and after the psychosocial stress test in boys with high-functioning autism spectrum disorders (hfASD) (95% confidence intervals).

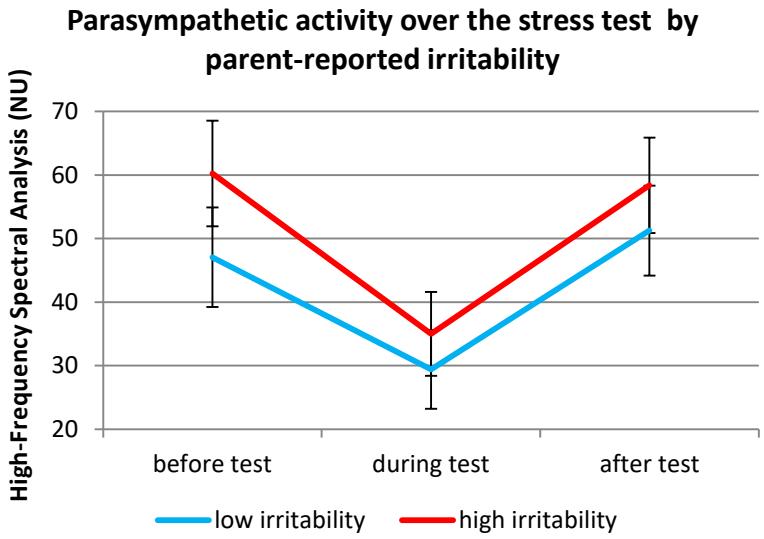


3.4.5.3 Heart rate variability: High-frequency spectral analysis

Parent report. We found a main effect of irritability, $F(1,32)=4.68$, $p=.038$, $\eta_p^2=.127$. Boys with high parent-reported irritability displayed overall higher parasympathetic modulation compared to those with low parent-reported irritability (Figure 3.5). However, this effect was no longer significant after parent-reported anxiety was added into the model as a covariate. Instead, there was a main effect of parent-reported anxiety, $F(1,31)=4.50$, $p=.042$, $\eta_p^2=.127$. Boys with high parent-reported anxiety displayed higher parasympathetic modulation compared to boys with low parent-reported anxiety.

Self report. No significant effects of self-reported irritability or anxiety on parasympathetic activity were found.

Figure 3.5. The relation between parent-reported irritability (median split, low vs. high) and parasympathetic activity before, during and after the psychosocial stress test in boys with high-functioning autism spectrum disorders (hfASD) (95% confidence intervals)



3.4.5.4 Heart rate variability: LF/HF ratio analysis

Parent report. We found no effects of parent-reported irritability on sympathetic activity. There was an independent main effect of parent-reported anxiety [$F(1,37)=4.74$, $p=.036$, $\eta_p^2=.114$], with boys rated as highly-anxious by their parents displaying lower sympathetic activity.

Self report. No significant effects of self-reported irritability or anxiety on sympathetic activity were found.

3.4.6 Irritability and physiological responses to stress in hfASD: Piecewise regression models

We used piecewise regression models to analyse the physiological response profiles of cortisol and HR across all time points of the PST. As explained in the Methods section, we fitted three-piece linear mixed models using two knot points, one at just before the initiation of the PST and the other corresponding to the peak/nadir of the stress response profile. The results complemented our ANOVAs and provided a stringent test of differences between physiological response profiles of adolescents with hfASD who were low vs. high on irritability.

3.4.6.1 Cortisol reactivity

A piecewise regression model was fitted to *log* cortisol data across each of the rest, stress and recovery phases of the PST. Importantly, as can be seen in Table 3.3 (page 102), there were no significant group differences in either the rest or the recovery phase slopes, for both self- and parent-reported irritability.

Parent report. In the transition from rest to stress, those rated as highly irritable by their parents had a significantly blunted cortisol response slope compared to those low on irritability, even after adding parent-reported anxiety into the model as a covariate (β = -0.27, p = .001). These results mirror the significant time by irritability interaction obtained using a repeated measures ANOVA reported in Section 3.4.5.1.

Self report. There were significant mean differences between boys who rated themselves as high vs. low on irritability, both at the first knot point (pre-stress; β_0 = -0.29, p = .04) and at the second knot point (post-stress, β_0 = -0.30, p = .04), with those high on irritability showing lower mean cortisol values. This is consistent with the main effect of self-reported irritability reported in Section 3.4.5.1. However, these mean differences were marginally no longer significant after adding anxiety into the model as a covariate.

3.4.6.2 Heart rate

Another piecewise regression model was fitted to the time-series mean HR data across the rest, stress and recovery phases of the psychosocial stress test. Similarly to cortisol findings, we did not find significant group differences in either the rest or the recovery phase slopes, for both self- and parent-reported irritability (see Table 3.3).

Parent report. In the transition from rest to stress, those rated as highly irritable by their parents had a significantly blunted HR response slope compared to those low on irritability (β =

-3.27, p = .03). The results remained significant after adding parent-reported anxiety into the model as a covariate (β = -3.27, p = .03). Boys who scored high on irritability also displayed lower mean HR at the second knot point (post-stress) compared to those low on irritability (β_0 = -7.98, p = .01). This effect remained significant after adding self-reported anxiety into the model as a covariate (β_0 = -7.65, p = .03).

Self report. No significant intercept or slope differences were found.

Table 3.3. Piecewise regression model statistics for cortisol and heart rate responses to psychosocial stress in boys with high-functioning ASD.

		Rest slope difference				**Mean difference at Pre-stress (Knot point: just before stressor)**				**Stress Slope Difference**				**Mean difference Post stress (Knot Point: peak stress response)**				**Recovery Slope Difference**			
	Cortisol	Coef.	SE	*p*	[95% CI]	Coef.	SE	*p*	[95% CI]	Coef.	SE	*p*	[95% CI]	Coef.	SE	*p*	[95% CI]	Coef.	SE	*p*	[95% CI]
parent report	high vs. low irritability	0.06	0.05	.29	-.05, .16	0.08	0.12	.48	-.15, .31	-0.27	0.08	**.001**	-.43, -.11	-0.19	0.12	.12	-.43, .05	0.09	0.05	.10	-.02, .19
	… covarying for anxiety	0.06	0.05	.29	-.05, .16	0.07	0.13	.57	-.18, .32	-0.27	0.08	**.001**	-.43, -.11	-0.20	0.13	.12	-.46, .06	0.09	0.05	.10	-.02, .19
self report	high vs. low irritability	-0.06	0.07	.38	-.21, .08	-0.29	0.14	**.04**	-.57, -.009	-0.02	0.12	.89	-.24, .21	-0.30	0.15	**.04**	-.60, -.008	0.03	0.07	.68	-.11, .18
	… covarying for anxiety	-0.06	0.07	.40	-.21, .08	-0.29	0.15	.05	-.58, .001	0.02	0.12	.89	-.22, .25	-0.27	0.16	.08	-.58, .04	0.02	0.08	.78	-.13, .17
	Heart Rate																				
parent report	high vs. low irritability	1.65	1.54	.28	-1.37, 4.67	-4.71	3.23	.15	-11.03, 1.62	-3.27	1.49	**.03**	-6.19, -.36	-7.98	3.21	**.01**	-14.26, -1.70	1.57	0.81	.05	-.006, 3.15
	… covarying for anxiety	1.65	1.54	.28	-1.37, 4.67	-4.38	3.48	.21	-11.21, 2.44	-3.27	1.49	**.03**	-6.18, -.36	-7.65	3.46	**.03**	-14.44, -.87	1.57	0.81	.05	-.006, 3.15
self report	high vs. low irritability	-0.23	1.78	.90	-3.72, 3.26	0.17	3.92	.96	-7.50, 7.85	-1.17	1.72	.50	-4.55, 2.21	-1.00	3.89	.80	-8.62, 6.63	-0.96	0.94	.31	-2.81, .89
	… covarying for anxiety	-0.35	1.84	.85	-3.96, 3.26	0.74	4.07	.86	-7.23, 8.72	-0.95	1.78	.60	-4.44, 2.55	-0.20	4.04	.96	-8.13, 7.73	-0.95	0.98	.33	-2.87, .96

SE, standard error. *CI*, confidence interval. *Rest slope* encompassed all time points before the stressor. *Stress slope* consisted of two time points: just before stressor and peak stress response. *Recovery Slope* started at peak stress response and encompassed all remaining time points.

3.5 Discussion

We showed that irritability can be measured reliably in boys with hfASD using a concise scale, and found a relation between irritability and physiological markers of stress response. Lower cortisol levels were observed in boys with high compared to low self-reported irritability; and lower HR was noted in boys with high compared to low parent-reported irritability. This was accompanied by a dampened physiological response to the experimental stressor in those with high parent-reported irritability. The results were consistent across two statistical approaches used.

Measurement of irritability in hfASD. Irritability showed high internal consistencies for both the parent- and self-reported scales. Item frequency pattern for both parent- and self-reported irritability in boys with hfASD mirrored that of boys with SMD. Consistent with previous results in TD children (Stringaris, Goodman, et al., 2012), we found a strong correlation between parent- and self-reported irritability in boys with hfASD. Finally, both boys with hfASD and their parents found the impairment due to irritability to be directly proportional to the level of irritability symptoms. This is consistent with previous studies showing that children with ASD can be as good as their parents in reporting on certain aspects of their psychopathology (Knott et al., 2006; Ozsivadjian & Knott, 2011), in particular when reporting on negative outcomes of difficulties with social interaction and temper management (Knott et al., 2006). Similarities between ARI scores in the SMD and hfASD groups suggest that irritability may be a critical dimension of the ASD phenotype. Additionally, the fact that irritability symptoms were impacting the lives of boys with hfASD argues for irritability to be a target for clinical interventions as in highly-irritable TD children (Scott & O'Connor, 2012).

Mechanisms of irritability in hfASD. We investigated whether irritability was associated with anxiety and stress-response in boys with hfASD. Parent- but not self-reports of irritability and anxiety were positively correlated, which may be due to introspection difficulties or alexithymia in the hfASD sample, a condition characterised by difficulties in identifying and describing emotions (Silani et al., 2008). Future studies should examine this possibility, since introspection difficulties and alexithymia were not measured in our study. However, this seems unlikely based on similar parent- and self-reporting on irritability in our hfASD group and the fact that boys with hfASD found the PST as stressful as TD boys did. An alternative possibility is that the hfASD boys in our sample were able to distinguish between their own feelings of anxiety and irritability better than their parents did.

Cortisol findings. The stress-induced increase in cortisol levels in hfASD was smaller than the increase in TD boys, despite an equally strong rise in subjective stress response across both groups. Boys with hfASD reporting high levels of irritability had significantly lower levels of cortisol; also boys who were rated as highly-irritable by their parents showed blunted cortisol

reactivity to stress. Low overall cortisol levels and dampened cortisol reactivity to psychosocial stress are often reported in children with ASD (Lanni et al., 2012; Levine et al., 2012), although the literature is inconsistent (Jansen et al., 2003) and cortisol levels in hfASD are within the normal range. Interestingly, lower cortisol levels throughout a psychosocial stress test have been previously reported in TD boys with disruptive behaviour disorders (van Goozen et al., 2000), and ODD (van Goozen et al., 1998)—both characterised by irritability (Krieger et al., 2013; Stringaris & Goodman, 2009b). Together with evidence for blunted cortisol response to psychosocial stress in TD children with externalising symptoms (Hartman et al., 2013), it is possible that irritability has a similarly blunting effect on cortisol stress-response in hfASD boys as it does in TD youth. However, low plasma levels of cortisol also feature in PTSD (Yehuda, 2001), a disorder where both irritability and anxiety are prominent (Stoddard et al., 2014). We found no independent contribution of anxiety to cortisol stress responses, consistent with a previous psychosocial stress study in boys with hfASD (Simon & Corbett, 2013). One possibility is that highly-irritable adolescents with hfASD are particularly liable to experience chronic stress. This is consistent with studies that reported decreased cortisol responses to stress in long-lasting internalising psychopathology including PTSD (Yehuda, 2001; Yehuda & Seckl, 2011) and chronic adolescent depression (Booij et al., 2013). Also in healthy children, Armbruster and colleagues showed that cortisol increase in response to the TSST was significantly blunted as the number of stressful life events increased (β = -0.19) (Armbruster et al., 2012). Future studies, longitudinal in nature, should investigate whether chronic stress contributes to the blunting of cortisol stress response in children with ASD. This is of particular interest given that toddlers with ASD display *increased* cortisol reactivity to stress compared to TD toddlers in response to the Strange Situation (caregiver separation) procedure (Naber et al., 2006), suggesting that cortisol stress reactivity in the ASD population may be downregulated over time.

Heart rate findings. Although for parent-reported irritability the pattern of HR results was similar to our findings with cortisol, the effects became non-significant after adding parent-reported anxiety into the model. Instead, boys with high parent-reported anxiety displayed dampened HR reaction to stress compared with boys with low parent-reported anxiety. This pattern of HR reactivity may reflect stress-induced physiological withdrawal of boys with hfASD who were rated as highly-anxious, based on our previous findings where children with ASD and anxiety showed reduced HR responsiveness to stress that was significantly related to anxiety severity (Hollocks et al., 2014). An alternative view could be that cortisol and HR responses to stress are qualitatively different, since HPA axis reactivity has a slower onset than the sympathetic system (Bauer, Quas, & Boyce, 2002). However, our HRV analyses revealed no relation between irritability and sympathetic system activity. Instead, irritability was related to parasympathetic activity, although its effect lost significance after adding anxiety into the model. No effects of self-reported irritability on HR and HRV were found, however it should be noted that we obtained fewer self- than parent-reports of irritability and a replication in a larger sample is needed.

Strengths and limitations. The strengths of this study include an experimental design and the use of multiple physiological measures: cortisol, HR, and HRV. All participants were medication-free, ensuring that the differences in physiology were unconfounded by treatment status. However, it is unclear whether the findings generalise to those individuals with ASD who take psychotropic medications. Similarly, it is unclear whether the findings would generalise to females. The study is limited by its modest sample size and unequal sample sizes between the groups. Due to the lack of variance in irritability scores among TD participants who took part in the PST, we were unable to compare the effects of irritability on physiological stress-responsiveness across boys with and without hfASD. A future study is needed for this purpose. Furthermore, while subjective stress ratings were collected immediately before and immediately after the stress phase of the PST, no rating was taken during the stress phase itself. Hellhammer and Schubert (2012) reported that ratings of anxiety, stress perception, and emotional insecurity were significantly higher when taken during the stress test rather than post-test. While we still found a significant increase in subjective stress from pre- to post-stressor in both boys with hfASD and TD controls, it is possible that the ratings may underestimate the peak of subjective stress response. Future studies wishing to characterise subjective stress response in more detail may benefit from including an additional self-reported stress rating between the figure drawing and speech phases of the test period. Motor movement controls were not thoroughly assessed in the HR analysis, although it is noteworthy that using the BioHarness^{TM} usually results in less movement artefacts compared to wire-based systems. ASD diagnoses could not be confirmed using structured assessments in all cases, although care was taken to limit the chance of false positives. Finally, our study did not assess the full range of comorbidities, in particular depression which is closely linked to irritability (Stringaris & Goodman, 2009a).

Clinical implications. Our results suggest that irritability can be reliably measured by both parent- and self-report in boys with hfASD and hence clinicians may benefit from using both parent- and child-rated scales to get a comprehensive view of the child's irritability. Second, irritability may shape physiological responses to stress in this population. This may have important implications for understanding the pathophysiological mechanisms of irritability in hfASD as well as for clinical practice. In TD youth, dampened cortisol responsiveness to stress predicted poorer treatment outcomes in disruptive behaviour disorder (van de Wiel, van Goozen, Matthys, Snoek, & van Engeland, 2004) and less improvement in depressive symptoms following CBT in children with a primary anxiety disorder (Dieleman, Huizink, Tulen, Utens, & Tiemeier, 2016). It therefore seems important to investigate the mechanisms of physiological underactivation in ASD during stress and clarify the role of irritability and anxiety in the process.

Chapter 4 – Disentangling the autism-anxiety overlap: fMRI of reward processing in a community-based longitudinal study

4.1 Abstract

Up to 40% of youth with ASD also suffer from anxiety (Simonoff et al., 2008) and this comorbidity is linked with significant functional impairment. However, the mechanisms of this overlap are poorly understood. We investigated the interplay between ASD traits and anxiety during reward processing, known to be affected in ASD, in a community sample of 1472 adolescents (mean age 14.4 years) who performed a modified monetary incentive delay task as part of the Imagen project. Blood-oxygen-level dependent (BOLD) responses to reward anticipation and feedback were compared using a 2x2 ANOVA (ASD traits: low/high, anxiety symptoms: low/high), controlling for plausible covariates. Additionally, we used a longitudinal design to assess whether neural responses during reward processing predicted anxiety at two-year follow-up. High ASD traits were associated with reduced BOLD responses in dorsal prefrontal regions during reward anticipation and negative feedback. Participants with high anxiety symptoms showed increased lateral prefrontal responses during anticipation, but decreased responses following feedback. Interaction effects revealed that youth with combined ASD traits and anxiety, relative to other youth, showed high right insula activation when anticipating reward, and low right-sided caudate, putamen, medial and lateral prefrontal activations during negative feedback (all clusters $p_{FWE}<.05$). BOLD activation patterns during reward anticipation in right dorsal cingulate and right medial frontal gyrus predicted new-onset anxiety in participants with high but not low ASD traits. Our results reveal both quantitatively enhanced and qualitatively distinct neural correlates underlying the comorbidity between ASD traits and anxiety. Specific neural responses during reward processing may represent a risk factor for developing anxiety in youth with ASD and could serve as a marker that is specific to the overlap as such, rather than of any of the individual disorders.

4.2 Introduction

Anxiety is common in young people with ASD (Mandy et al., 2014; Mazefsky, Conner, & Oswald, 2010; Mikita et al., 2015; Simonoff et al., 2012; Van Steensel et al., 2011) and in young people with sub-diagnostic autistic traits (Hallett et al., 2010, 2012). Comorbid anxiety causes significant functional impairment for young people with ASD (Mattila et al., 2010; Simonoff et al., 2008) and impacts on the quality of life of their families (J. A. Kim et al., 2000). However, the mechanisms of this association are poorly understood. While considerable research examined neural correlates of anxiety in adolescents, few studies examined these correlates in children with symptoms of ASD. Here we investigate whether aberrations in reward processing underlie the co-occurrence of ASD traits and anxiety and whether they predict the *new* onset of anxiety in youth with ASD traits.

Reward processing has been proposed to be central to ASD (Dichter & Adolphs, 2012; Gaigg, 2012), with aberrant processing of primary (Cascio et al., 2012), social (Delmonte et al., 2012; Demurie et al., 2011), and monetary rewards (Kohls et al., 2013) reported in children and young people with ASD. Furthermore, studies in youth with ASD reported associations between brain activations during reward processing and ASD traits such as social communication difficulties (Damiano et al., 2015) and restricted and repetitive behaviours (Cascio et al., 2014). Some (Delmonte et al., 2012; McPartland, Crowley, et al., 2012; Stavropoulos & Carver, 2014) but not all studies (Kohls et al., 2013) suggested that the extent to which reward processing in youth with ASD differs from typically developing controls may depend on reward type.

Surprisingly, however, the question whether these reward aberrations are inherent to ASD symptoms or related to disorders that co-occur with ASD remains unanswered. This is a key question considering that less than 10% of children with ASD are free of any concomitant disorders according to some studies (Lundstrom et al., 2015; Salazar et al., 2015). Anxiety disorders and behavioural difficulties are consistently identified as the most common comorbidities in youth with ASD (Leyfer et al., 2006; Simonoff et al., 2008). These comorbid disorders are associated with aberrant reward processing in their own right, and therefore could influence reward processing in youth with ASD. Young people with anxiety show disrupted frontostriatal activation when anticipating reward (Bar-Haim et al., 2009; Guyer, Choate, Detloff, et al., 2012; Guyer et al., 2006) and when receiving reward feedback (Helfinstein et al., 2011). Youth with behavioural difficulties, in particular ODD symptoms and irritability, often show aberrant responses when rewards fail to appear, perhaps due to frustrative nature of negative outcomes (Bjork et al., 2010; Deveney et al., 2013).

The aim of the present study is to use fMRI to disentangle the interplay of ASD traits and anxiety during reward processing. To enable the investigation of these factors, we use a large, community-based sample of adolescents with high vs. low levels of ASD traits and anxiety

symptoms, who completed a widely-used, modified monetary incentive delay (MID) fMRI reward task. Our aim is to distinguish between two important models of comorbidity. One model – additive comorbidity – assumes that neural correlates of the comorbidity simply reflect the co-occurrence of neural mechanisms seen with each ASD traits and anxiety separately (Banaschewski et al., 2007; Caron & Rutter, 1991). In the other model – independent nosology – the neural correlates of the comorbidity are unique, i.e., not seen with any of the two disorders. This distinction is crucial from an aetiological and clinical perspective, as finding unique correlates would suggest that the comorbidity might represent a separate nosological process, what has been termed a "third independent disorder" (Neale & Kendler, 1995). In addition, our aim is to establish the potential predictive value of neural correlates of comorbidity. Using a longitudinal design, we examine whether neural correlates found in people with concurrent ASD trait-anxiety comorbidity can be used to predict the likelihood of new onset anxiety in those with high ASD traits only. We achieve this aim in two steps:

First, we test the independent influence of each ASD traits and anxiety on brain correlates of reward processing, and also to examine possible interaction effects between ASD traits and anxiety. The latter is particularly important in order to assess whether combined ASD traits and anxiety is associated with distinct etiological mechanisms (Banaschewski et al., 2007).

Second, we assess whether the brain activations found in our cross-sectional interaction analyses represent a biomarker that also predicts *successive comorbidity* (Angold et al., 1999) between ASD traits and anxiety, that is whether such brain activations predict the new onset of anxiety in those with high ASD traits. To achieve this, we run regression models with anxiety at two-year follow-up as the outcome, and brain activations (relevant to the comorbid group) as the predictor of interest, separately for participants with low vs. high ASD traits, controlling for baseline anxiety. To capture major elements of reward processing, we enquire about two key stages: reward anticipation and reward feedback. The former is often associated with heightened frontostriatal activation in youth with anxiety symptoms (Bar-Haim et al., 2009; Guyer, Choate, Detloff, et al., 2012; Guyer et al., 2006; Pine, 2007); whereas negative feedback is likely to elicit frustration, possibly related to irritability/ODD symptoms that are common in ASD (Mandy et al., 2014; Mikita et al., 2015) but also in anxiety (Stoddard et al., 2014). Consequently, in line with the aims of this thesis (to investigate the joint effects of the commonly co-occurring anxiety and irritability) we also test whether irritability symptoms are related to neural activation changes following negative feedback in youth with ASD traits as found in young people with SMD (Deveney et al., 2013). For completeness, we also examine positive reward feedback, since youth with ASD tend to show reduced responsiveness to rewards in fMRI studies (Delmonte et al., 2012; Kohls et al., 2013). By using a community sample we avoid the risk of referral bias typical of clinical or convenience samples, a pertinent issue when investigating comorbidity (Angold et al., 1999; Caron & Rutter, 1991). We also follow the emerging evidence that ASD traits, but also the

mechanisms underlying them, fall on a continuum within the general population (Blanken et al., 2015; Constantino & Todd, 2003).

4.3 Methods

4.3.1 Participants

Data were obtained from the Imagen database established across eight sites in France, United Kingdom, Ireland, and Germany, which includes 2,223 adolescents recruited in schools. We used data from the first (age around 14 years) and second waves (age around 16 years) of Imagen. Recruitment and assessment procedures were described in detail previously (Schumann et al., 2010). All local ethics research committees approved the study. Written consent was obtained from a parent or guardian, and verbal assent was obtained from the adolescent. Any adolescents with IQ<70 were excluded from this study. After quality control for neuroimaging and behavioural tests, final sample sizes were 1472 for reward anticipation, 1601 for negative feedback and 1726 for positive feedback. Differences in sample sizes across conditions are due to some fMRI contrasts being non-estimable for some participants at the first-level analysis stage. Reasons included: incomplete fMRI runs, questionable values in behavioural csv files, and algorithmic failure of a previous step (e.g., preprocessing, dicom file conversion). Sample characteristics were highly similar across all three reward conditions (see Table 4.1 and Table 4.2 on pages 118 and 120).

4.3.2 Measures

IQ was estimated with the Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV) (Wechsler, 2003), in wave one and entered into Psytools (Delosis, London), an online computer platform. Two standardised indices were calculated from the WISC subtests applied during neuropsychological testing for Imagen: Verbal Comprehension (derived from Vocabulary and Similarities subtests) and Perceptual Reasoning (derived from Block Design and Matrix Reasoning subtests).

ASD traits were assessed in wave one using the ASD section from the Development and Well-Being Assessment (DAWBA) (R. Goodman, Ford, Richards, Gatward, & Meltzer, 2000); www.dawba.info), a parent-reported, self-administered structured diagnostic interview with: 15 questions about social difficulties, 14 questions about restricted, repetitive behaviours and

interests, and three questions about language development to assess DSM-IV-defined ASD symptoms. The DAWBA ASD section contains a "skip rule" whereby all parents are asked some initial questions, but only a subset of parents are asked the remaining, more detailed questions. The skip rule is used unless the following criteria are met: the presence of possible ASD symptoms in the first 3 years of life that have not resolved (assessed by initial screening questions in the ASD section); a Strengths and Difficulties Questionnaire (SDQ, see below) profile with high peer problems and a low prosocial score; or a Social Aptitudes Scale (SAS, see below) score of 12 or below. The full set of questions was administered to 104 (7.1%) parents of participants with scans available for the reward anticipation condition; to 117 (6.8%) and 115 (7.2 %) for positive and negative feedback, respectively. In addition, parents who completed the full DAWBA ASD section were asked to complete an impact supplement asking about distress due to ASD-related difficulties in the past 12 months and impact of the difficulties on family life, friendships, classroom learning and leisure activities. A score of 2 or above on this scale indicates "high" levels of impairment. The diagnostic algorithm derived from the DAWBA ASD module shows strong agreement with that from the Autism Diagnostic Interview-Revised (ADI-R) (Colvert et al., 2015; Lord et al., 1994) and has a high predictive value for ASD diagnoses in community settings (McEwen et al., 2016). In line with the newest characterization of ASD (APA, 2013), we classified participants as having "high" levels of ASD traits if their parents/carers reported three or more symptoms on the social difficulties subscale and three or more symptoms on the restricted and repetitive behaviours subscale of the DAWBA ASD section. Based on this criterion close to 5% of the sample had high ASD traits (Table 4.1), consistent with prevalence of clinically relevant autistic traits reported in previous population-based studies (e.g., (Kothari et al., 2013).

The Social Aptitudes Scale (SAS) (Liddle et al., 2008), http://www.dawba.com/SAS) was used as an additional measure of social skills in the sample. The SAS is a ten-question, parent-reported scale that taps social aptitude skills that require ability to read social and emotional cues rapidly in complex situation to guide socially-skilled behaviour. For each item, parents rated their child as "a lot worse than average", "a bit worse than average", "about average", "a bit better than average" or "a lot better than average" compared to children of the same age. The total sum of items ranges between 0 and 40 with lower scores marking lower social aptitude skills. The internal consistency of the scale is very good with Cronbach's alpha of 0.88; the SAS also shows good validity as it is positively correlated with the prosocial subscale of the SDQ (r=0.42, p<.001) (Liddle et al., 2008).

Anxiety, ODD and depression prevalences were estimated based on the established and widely-used DAWBA computer algorithm (A. Goodman, Heiervang, Collishaw, & Goodman, 2011; R. Goodman et al., 2000), which indicates the probability of receiving a DSM-IV-defined diagnosis based on answers provided during interview. Due to few participants with high ASD traits scoring at band 3 or above (n<10 for each anxiety disorder), we chose band 2 as a categorical

cut-off point for relevant psychiatric symptoms. Participants were classified as having "any anxiety" if they scored at band 2 or above for at least one DSM-IV anxiety disorder (separation anxiety, specific phobia, social anxiety, GAD, agoraphobia, panic disorder, OCD, post-traumatic stress disorder); this algorithm identified around 24% of the sample (see Table 4.2).

Emotional symptoms were assessed using the parent-reported emotional symptoms subscale from the Strengths and Difficulties Questionnaire (SDQ) (R. Goodman, 2001). The scale includes three questions about anxiety, one question about somatic symptoms, and one about low mood. A score of 5 and above indicates substantial risk of clinically significant emotional problems (R. Goodman, 2001) and was used as a cut-off. We used emotional symptoms instead of "any anxiety" in confirmatory analyses (see below) to ensure that our findings were not measure-specific.

Irritability symptoms in the previous six months were measured with three items pertaining to the irritability dimension from the DAWBA section on ODD, as used previously (Stringaris & Goodman, 2009b). Parents were asked to rate their children against others of the same age based on whether the child has: (1) "had temper outbursts", (2) "been touchy or easily annoyed", and (3) "been angry and resentful". There were three possible response categories for each item: "no more than others" (scored 0), "a little more than others" (scored 1), and "a lot more than others" (scored 2).

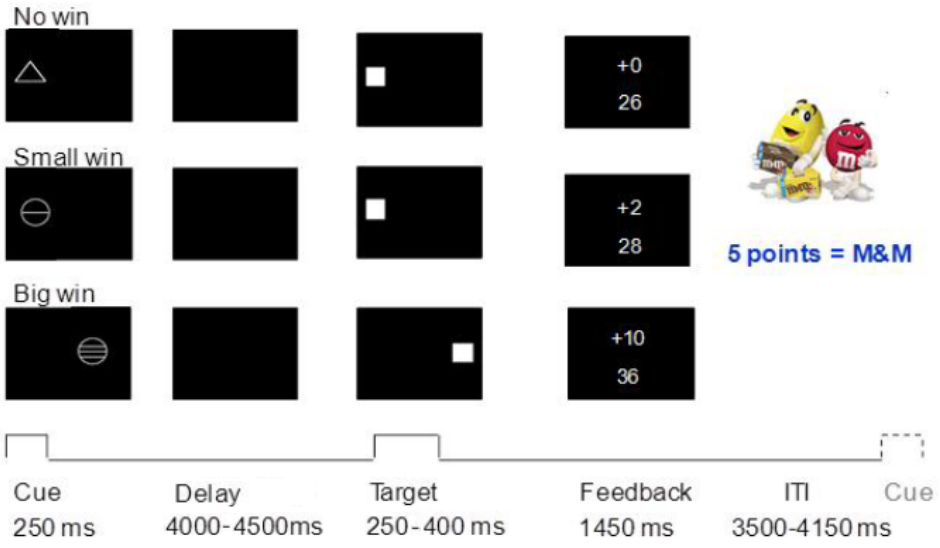
Additional relevant symptoms of hyperactivity, conduct problems, and functional impairment were assessed using the parent-reported SDQ (R. Goodman, 2001). An SDQ impact score of 2 or above indicates "high" risk of clinically significant functional impairment based on prevalences reported in an epidemiological study (R. Goodman, 2001).

4.3.3 Modified Monetary Incentive Delay (MID) Task

In wave one, the participants performed a modified version of the well-established MID task (Knutson, Adams, Fong, & Hommer, 2001; Knutson et al., 2000; Stringaris et al., 2015) to study neural responses to reward. This event-related task consisted of 66 ten-second trials. In each particular trial, participants were presented with one of three cue shapes (cue, 250 ms) denoting whether a target (a white square) would subsequently appear on the left or right side of the screen and whether 0, 2 or 10 points could be won in that particular trial (Figure 4.1). After a variable delay (4000-4500 ms), participants were instructed to respond by pressing a button with their left or right index finger as soon as the target appeared. Feedback on whether and how many points were won during the trial was presented for 1450 ms after the response. Using a tracking algorithm, task difficulty (i.e., target duration that varied between 250 and 400 ms) was individually adjusted such that each participant responded successfully on around 66% of trials. Participants had first completed a practice session outside the scanner (for around 5 minutes),

during which they were instructed that after the scanning session concluded, the points won on the task would be converted into chocolate sweets (one M&M for every 5 points). Since the participants did not receive food immediately following successful performance on each trial, but rather after the scanning session had finished, the modified MID did not constitute of primary reward task. Functional MRI BOLD responses were measured during reward anticipation and reward feedback. The following three task conditions were used in this study: reward anticipation (anticipation of large win versus anticipation of no win), receipt of negative feedback (feedback of missed large win versus feedback of missed no win) and positive feedback (feedback of hit large win versus feedback of hit no win). Task presentation and recording of the behavioural responses were performed using Visual Basic 2005 with .NET Framework Version 2.0, and the visual and response grip system from Nordic Neuro Lab (Bergen, Norway).

Figure 4.1. Outline of the stages of the modified Monetary Incentive Delay task.



4.3.4 Magnetic Resonance Imaging Data Acquisition and Preprocessing

Structural and functional MRI data were acquired at eight Imagen assessment sites with 3T MRI scanners from different manufacturers (Siemens, Munich, Germany; Philips, Best, The Netherlands; General Electric, Chalfont St Giles, UK; Bruker, Ettlingen, Germany). To address the difficulty of pooling data acquired on different scanners (since the scanners vary in terms of the availability and implementation of particular image-acquisition techniques), a set of parameters compatible with all scanners was devised and held constant across sites for each technique. Where manufacturer-specific choices had to be made (for example, the design of head coil), the best manufacturer-specific option was used at all sites with the same scanner type. The following quality control procedures were regularly implemented at each site: (1) The American

College of Radiology phantom is scanned to provide information about geometric distortions and signal uniformity related to hardware differences in radiofrequency coils and gradient systems, image contrast and temporal stability, and a custom phantom is scanned for diffusion-related parameters. (2) Several healthy volunteers are regularly scanned at each site to assess factors that cannot be measured using phantoms alone and at multiple sites to determine inter-site variability in structural and functional measures (for example, tissue contrast in raw MRI signal, tissue relaxation properties). More details about quality information can be found in Schumann et al (2010).

The scanning variables were specifically chosen to be compatible with all scanners. The same scanning protocol was used at all sites. High-resolution T1-weighted three-dimensional structural images were acquired for anatomical localization and co-registration with the functional time series. The T1-weighted magnetization prepared gradient echo sequence (MPRAGE) images were obtained using a modified protocol based on the ADNI project (http://adni.loni.ucla.edu/methods/documents/mri-protocols/). The images comprised 160 slices with 1.1 × 1.1 × 1.1 mm3 voxel size, field of view frequency 28 cm. Functional MRI BOLD images were acquired with a gradient-echo, echo-planar imaging sequence. For the modified Monetary Incentive Delay task, 300 volumes were acquired for each subject. Each volume consisted of 40 slices aligned to the anterior commissure- posterior commissure line (2.4mm slice thickness, 1mm gap) acquired in a descending order. The echo time was optimised (echo time = 30 msec, repetition time = 2200 msec) to provide reliable imaging of subcortical areas.

Functional MRI data were preprocessed using the Statistical Parametric Mapping suite (SPM 8, Functional Imaging Laboratory, University College London, www.fil.ion.ucl.ac.uk/spm). Time series data were slice-time corrected using the first slice as the reference for interpolation, and then corrected for movement (spatial realignment) to the first volume. Time series data were then non-linearly warped on the MNI space, using a custom EPI template and smoothed with a Gaussian kernel of 5mm Full-Width Half Maximum (FWHM).

At the first level of analysis, the model contained the onset of each cue and each feedback presentation. This enables separate analyses of reward anticipation and reward feedback conditions. Each trial (e.g., reward feedback large win) was convolved using the SPM default Hemodynamic Response Function. Estimated movement parameters were added to the design matrix in the form of 18 additional columns (3 translations, 3 rotations, 3 quadratic and 3 cubic translations, and each 3 translations with a shift of ±1 TR).

4.3.5 Statistical analyses

Behavioural performance. We first tested whether participants were motivated by the potential of winning a reward. As a proxy of task engagement, we compared mean response accuracy in "no win" and "big win" trials using a paired-samples t-test, separately for participants with low and high ASD traits.

Imaging analyses were performed using SPM 8. Analyses were performed at an *a priori* voxel threshold of $p<0.01$ and cluster threshold of $p<0.05$ with family-wise error (FWE) correction. Gender, handedness, site of scanning, WISC Verbal Comprehension, and WISC Reasoning were included as covariates in all analyses, in line with previous studies (Delmonte et al., 2012; Graham et al., 2010; Reiss, Abrams, Singer, Ross, & Denckla, 1996). All findings are reported at whole-brain level.

Effects of ASD traits and anxiety on reward processing. We ran a 2x2 ANOVA [ASD traits: low vs. high; any anxiety: low (DAWBA bands 0/1) vs. high (band 2 or above)] to test for main effects of ASD traits and anxiety, and an ASD-by-anxiety interaction, separately for reward anticipation, negative and positive feedback conditions. Where an ASD-by-anxiety interaction was found, we ran follow-up t-tests to assess between-groups differences in brain activations.

Effects of ODD and depression. We repeated the above ANOVAs twice, first adding ODD and then depression into the model as potential confounders.

Effects of emotional problems. To ensure that our main findings were not measure-specific, we ran confirmatory ANOVAs and t-tests using the SDQ emotional symptoms subscale (cut-off at 5) instead of DAWBA-defined "any anxiety".

Effects of different anxiety categories. Where an ASD-by-anxiety interaction was found, we additionally tested whether reward processing in youth with high ASD traits was associated with the number of different anxiety disorders, or with specific types of anxiety disorders. First, we ran a multiple regression with the number of anxiety disorders at band 2 or above as a predictor, to test whether the pattern of brain activations during reward processing is associated with anxiety quantitatively. Then, we conducted t-tests to compare brain activations between participants with different types of anxiety. Due to sample size limitations (see Table 4.3), we restricted these analyses to GAD and social anxiety.

Effects of irritability. To test whether neural responses to not receiving a reward were associated with irritability symptoms, we conducted a multiple regression in SPM with the sum of ODD irritability item scores as predictor, and whole-brain activation maps from the negative feedback condition as the outcome. Regressions were run separately for participants with high and low ASD traits.

Longitudinal predictions. Our second aim was to assess whether the brain activations found in cross-sectional interaction analyses above are (a) a phenotypic manifestation of the co-

occurrence between ASD traits and anxiety, or (b) whether they represent a biomarker that predicts not only cross-sectional, but also successive comorbidity between ASD traits and anxiety. To test this we conducted logistic regression analyses with anxiety (low vs. high) at two-year follow up as the outcome and brain activations found in our cross-sectional interaction effects as predictors of interest. Analyses were run in Stata 11 (StataCorp, 2009) and the regions-of-interest (ROIs) were extracted from the contrast maps for a given reward condition, using MarsBaR toolbox in SPM based on the WFUPickAtlas toolbox definitions (Maldjian, Laurienti, Kraft, & Burdette, 2003). To ensure that the ROIs predicted new onset of anxiety, baseline anxiety status was added to the model as an additional predictor. We also added baseline ASD traits (continuous variable) to the model, to ensure that the predictions were not driven by the severity of ASD-specific impairments. To assess the specificity of brain predictions, regressions were run separately within low and high ASD traits groups. If an ROI predicted new-onset anxiety in one group but not the other, a regression model was run across the whole sample with an interaction term between ROI activations and ASD traits (low vs. high) to identify the strength of a possible interaction.

Core ASD. As a plausibility check, to test whether the neuroimaging results found within the high ASD traits group held in participants who met more strict ASD criteria, we re-ran the relevant cross-sectional t-tests within a smaller sample of participants who met ASD traits criteria based on the DAWBA ASD algorithm at band 2 or above (n=27-31 / 1.8-1.9% depending on reward condition, see Table 4.16). We did not run corresponding ANOVAs due to the small sample size of the core ASD traits group.

4.4 Results

4.4.1 Participant characteristics

ASD symptoms and demographics. As expected, participants with high ASD traits scored significantly higher on all subscales of the DAWBA ASD section and significantly lower on the SAS compared to those with low ASD traits, who also had a higher proportion of males, close to the reported 3:1 male:female ratio (Table 4.1) (Baird et al., 2006). While the two groups did not differ in age or performance IQ, youth with high ASD traits scored lower on verbal comprehension, and differences in both verbal and performance IQ were found when comparing four groups of participants, with the comorbid ASD + anxiety group scoring the lowest on both IQ measures (Table 4.1). With regards to impact due to ASD symptoms in participants with high

ASD traits, this was substantial. Of the participants whose parents completed this section, 20/35 (57%) scored above 2 on the impact scale[3].

Other psychopathology. As shown in Table 4.2, participants with high ASD traits scored higher on hyperactivity, emotional, and conduct problems, and showed higher functional impairment compared to participants with low ASD traits. They were also more likely to display symptoms of anxiety, depression, and ODD. These between-group differences persisted at two-year follow-up. Among anxiety symptoms, social and generalised anxiety were the most prevalent within the high ASD traits group (Table 4.3).

[3] Data for participants with high ASD traits who had scans available for the reward anticipation phase. For negative and positive feedback, the proportion was 22/38 (58%).

Table 4.1. Demographic characteristics of the sample and ASD symptoms (data from wave 1).

	Total: Low ASD traits	Total: High ASD traits	None	Anxiety only	ASD traits only	ASD traits & anxiety
(a) Reward anticipation						
n	1402	70	1076	326	40	30
Male gender	657 (46.9%)	47 (67.1%)**	527 (49.0%)^{a}	130 (39.9%)^{b}	28 (70.0%)^{c}	19 (63.3%)^{a}
Age in years	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4	14.5 ± 0.4
WISC Verbal	112.0 ± 14.7	108.1 ± 15.1*	112.8 ± 14.6^{a}	109.2 ± 14.7^{b}	110.2 ± 16.2^{a,b}	105.3 ± 13.4^{b}
WISC Reasoning	108.1 ± 13.9	108.8 ± 14.4	108.6 ± 13.9^{a}	106.5 ± 13.8^{b}	113.3 ± 13.4^{a}	102.9 ± 13.7^{b}
ASD symptoms (DAWBA)						
total	0.3 ± 1.6	17.4 ± 5.5***	0.3 ± 1.2^{a}	0.6 ± 2.4^{b}	16.6 ± 5.3^{c}	18.4 ± 5.6^{d}
social difficulties	0.2 ± 1.2	9.9 ± 4.1***	0.1 ± 0.8^{a}	0.4 ± 1.9^{b}	9.4 ± 3.9^{c}	10.7 ± 4.4^{d}
repetitive behaviours	0.1 ± 0.5	6.8 ± 3.7***	0.1 ± 0.5^{a}	0.1 ± 0.6^{a}	6.5 ± 3.4^{b}	7.3 ± 4.0^{c}
language development	0.1 ± 0.3	0.6 ± 0.9***	0.1 ± 0.3^{a}	0.1 ± 0.3^{a}	0.7 ± 1.0^{b}	0.4 ± 0.8^{c}
Social Aptitudes Scale	24.8 ± 5.6	17.9 ± 7.4***	25.1 ± 5.4^{a}	23.6 ± 5.8^{b}	20.0 ± 6.7^{c}	15.2 ± 7.7^{d}
(b) Negative feedback						
n	1523	78	1162	361	43	35
Male gender	738 (48.5%)	54 (69.2%)***	595 (51.2%)^{a}	143 (39.6%)^{b}	30 (69.8%)^{a}	24 (68.6%)^{a}
Age in years	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4	14.5 ± 0.4
WISC Verbal	111.2 ± 14.7	107.0 ± 15.9*	112.0 ± 14.6^{a}	108.8 ± 15.0^{b}	109.5 ± 16.0^{a,b}	104.0 ± 15.5^{b}
WISC Reasoning	107.7 ± 14.2	107.2 ± 15.6	108.3 ± 14.0^{a}	105.9 ± 14.6^{b}	112.6 ± 14.4^{a}	100.6 ± 14.2^{b}
ASD symptoms (DAWBA)						
total	0.3 ± 1.6	17.1 ± 5.5***	0.3 ± 1.3^{a}	0.6 ± 2.4^{b}	16.3 ± 5.5^{c}	18.2 ± 5.3^{d}
social difficulties	0.2 ± 1.2	9.1 ± 4.2***	0.1 ± 0.8^{a}	0.4 ± 1.9^{b}	9.4 ± 4.2^{c}	10.4 ± 4.2^{d}
repetitive behaviours	0.1 ± 0.6	6.6 ± 3.6***	0.1 ± 0.5^{a}	0.1 ± 0.7^{a}	6.1 ± 3.3^{b}	7.2 ± 3.9^{c}
language development	0.1 ± 0.3	0.6 ± 0.9***	0.1 ± 0.3^{a}	0.1 ± 0.3^{a}	0.7 ± 1.0^{b}	0.5 ± 0.9^{c}

Social Aptitudes Scale	24.8 ± 5.6	18.3 ± 7.4***	25.1 ± 5.5[a]	23.6 ± 5.7[b]	20.2 ± 6.6[c]	15.9 ± 7.8[d]
(c) Positive feedback						
n	1645	81	1256	389	44	37
Male gender	795 (48.3%)	57 (70.4%)***	639 (50.9%)[a]	156 (40.1%)[b]	32 (72.7%)[c]	25 (67.6%)[a,c]
Age in years	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4	14.5 ± 0.4
WISC Verbal	111.3 ± 14.8	107.2 ± 15.5*	112.2 ± 14.6[a]	108.5 ± 15.0[b]	110.1 ± 15.4[a,b]	103.8 ± 15.1[b]
WISC Reasoning	107.5 ± 14.1	107.5 ± 14.9	108.0 ± 14.0[a]	105.8 ± 14.4[b]	113.0 ± 13.2[a]	101.1 ± 14.5[b]
ASD symptoms (DAWBA)						
total	0.3 ± 1.5	17.4 ± 5.3***	0.3 ± 1.2[a]	0.6 ± 2.2[b]	16.8 ± 5.4[c]	18.0 ± 5.2[d]
social difficulties	0.2 ± 1.1	10.0 ± 4.1***	0.1 ± 0.8[a]	0.4 ± 1.8[b]	9.7 ± 4.0[c]	10.3 ± 4.2[c]
repetitive behaviours	0.1 ± 0.5	6.8 ± 3.6***	0.1 ± 0.5[a]	0.1 ± 0.6[a]	6.4 ± 3.4[b]	7.2 ± 3.8[c]
language development	0.1 ± 0.3	0.6 ± 0.9***	0.1 ± 0.3[a]	0.1 ± 0.3[a]	0.7 ± 1.0[b]	0.5 ± 0.9[b]
Social Aptitudes Scale	24.7 ± 5.6	17.9 ± 7.3***	25.0 ± 5.4[a]	23.5 ± 5.8[b]	19.7 ± 6.6[c]	15.7 ± 7.7[d]

*$p < .05$; **$p < .01$; ***$p < .001$.
a, b, c, d = different letters indicate a significant group difference from each other at $p<.05$ (Bonferroni corrected).

Table 4.2. Symptoms of psychopathology in the sample at waves 1 and 2.

	Total: Low ASD traits[4]	Total: High ASD traits[5]	None	Anxiety only	ASD traits only	ASD traits & anxiety
(a) Reward anticipation						
Baseline						
n	1402	70	1076	326	40	30
Continuous psychopathology (SDQ)						
emotional symptoms	1.8 ± 1.9	3.5 ± 2.9***	1.2 ± 1.3[a]	3.9 ± 2.0[b]	2.0 ± 1.9[c]	5.5 ± 2.8[d]
conduct problems	1.6 ± 1.5	2.9 ± 2.2***	1.4 ± 1.4[a]	2.0 ± 1.8[b]	2.3 ± 1.9[b]	3.8 ± 2.3[c]
hyperactivity	2.8 ± 2.2	4.5 ± 2.9***	2.6 ± 2.1[a]	3.5 ± 2.4[b]	3.5 ± 2.4[b]	5.9 ± 3.1[c]
impact	0.6 ± 1.3	2.2 ± 2.4***	0.4 ± 0.9[a]	1.2 ± 1.8[b]	1.0 ± 1.6[b]	3.8 ± 2.6[c]
Diagnostic categories						
any anxiety	326 (23.3%)	30 (42.9%)***	0[a]	326 (100%)[b]	0[a]	30 (100%)[b]
depression	44 (3.1%)	10 (14.3%)***	16 (1.5%)[a]	28 (8.6%)[b]	2 (5.0%)[a,b]	8 (26.7%)[c]
ODD	483 (34.5%)	43 (61.4%)***	301 (28.0%)[a]	182 (55.8%)[b]	23 (57.5%)[b]	20 (66.7%)[b]
2-year follow up						
n	1019	50	793	226	28	22
Male gender	465 (45.7%)	35 (70.0%)**	380 (48.0%)[a]	85 (37.6%)[b]	21 (75.0%)[c]	14 (63.6%)[a,b,c]
Continuous psychopathology (SDQ)						
emotional symptoms	1.6 ± 1.9	2.9 ± 2.9**	1.2 ± 1.5[a]	3.1 ± 2.3[b]	1.6 ± 2.2[a]	4.5 ± 3.0[c]
conduct problems	1.4 ± 1.4	2.0 ± 1.9*	1.3 ± 1.4[a]	1.7 ± 1.6[b]	1.6 ± 1.9[a,b]	2.5 ± 1.9[b]
hyperactivity	2.2 ± 2.0	3.6 ± 2.5***	2.1 ± 1.9[a]	2.6 ± 2.1[b]	2.8 ± 2.2[a,b]	4.7 ± 2.5[c]
impact	0.5 ± 1.4	1.3 ± 2.1*	0.4 ± 1.2[a]	1.1 ± 1.9[b]	1.0 ± 2.0[a,b]	1.6 ± 2.3[b,c]
Diagnostic categories						
any anxiety	196 (19.3%)	17 (34.0%)*	96 (12.1%)[a]	100 (44.2%)[b]	5 (17.9%)[c]	12 (54.5%)[b]
depression	36 (3.5%)	6 (12.0%)*	18 (2.3%)[a]	18 (8.0%)[b]	2 (7.1%)[b]	4 (18.2%)[b]
ODD	295 (29.0%)	23 (46.0%)*	201 (25.4%)[a]	94 (41.8%)[b]	10 (35.7%)[b]	13 (59.1%)[b]

[4] 1279 participants with low ASD traits provided neuroimaging data across reward anticipation, positive and negative feedback conditions.
[5] 65 participants with high ASD traits provided neuroimaging data across reward anticipation, positive and negative feedback conditions.

(b) Negative feedback						
Baseline						
n	1523	78	1162	361	43	35
Continuous psychopathology (SDQ)						
emotional symptoms	1.9 ± 1.9	3.5 ± 2.9***	1.2 ± 1.3[a]	4.0 ± 2.1[b]	1.8 ± 1.9[a]	5.6 ± 2.6[c]
conduct problems	1.6 ± 1.6	2.9 ± 2.1***	1.4 ± 1.4[a]	2.1 ± 1.8[b]	2.3 ± 1.8[b]	3.7 ± 2.3[c]
hyperactivity	2.8 ± 2.2	4.6 ± 2.9***	2.6 ± 2.1[a]	3.6 ± 2.4[b]	3.5 ± 2.3[b]	6.1 ± 2.8[c]
impact	0.6 ± 1.3	2.1 ± 2.4***	0.4 ± 1.0[a]	1.3 ± 1.9[b]	0.9 ± 1.5[b]	3.5 ± 2.5[c]
Irritability symptoms (DAWBA)	0.5 ± 1.1	1.7 ± 1.8***	0.4 ± 0.9 [a]	1.0 ± 1.4[b]	1.2 ± 1.7[b]	2.4 ± 1.7[c]
Diagnostic categories						
any anxiety	361 (23.7%)	35 (44.9%)***	0[a]	361 (100%)[b]	0[a]	35 (100%)[b]
depression	48 (3.2%)	13 (16.7%)***	20 (1.7%)[a]	28 (7.8%)[b]	3 (7.0%)[a,b]	10 (28.6%)[c]
ODD	534 (35.1%)	49 (62.8%)***	326 (28.1%)[a]	208 (57.6%)[b]	25 (58.1%)[b]	24 (68.6%)[b]
2-year follow up						
n	1124	55	872	252	30	25
Male gender	533 (47.4%)	37 (67.3%)**	437 (50.1%)[a]	96 (38.1%)[b]	21 (70.0%)[a]	16 (64.0%)[a]
Continuous psychopathology (SDQ)						
emotional symptoms	1.7 ± 1.9	3.0 ± 3.0**	1.3 ± 1.6[a]	2.9 ± 2.3[b]	1.8 ± 2.4[a]	4.4 ± 3.0[c]
conduct problems	1.4 ± 1.4	2.2 ± 2.0**	1.3 ± 1.4[a]	1.6 ± 1.6[b]	1.9 ± 2.1[a,c]	2.5 ± 1.9[c]
hyperactivity	2.3 ± 2.0	3.8 ± 2.5***	2.2 ± 1.9[a]	2.7 ± 2.1[b]	3.0 ± 2.2[a,b]	4.7 ± 2.4[c]
impact	0.5 ± 1.3	1.4 ± 2.2**	0.4 ± 1.1[a]	1.0 ± 1.8[b]	1.2 ± 2.2[b]	1.6 ± 2.3[b]
Diagnostic categories						
any anxiety	228 (20.3%)	21 (38.2%)**	116 (13.3%)[a]	112 (44.4%)[b]	7 (23.3%)[a,b]	14 (56.0%)[b]
depression	38 (3.4%)	7 (12.7%)**	20 (2.3%)[a]	18 (7.1%)[b]	3 (10.0%)[b]	4 (16.0%)[b]
ODD	322 (28.7%)	26 (47.3%)**	219 (25.1%)[a]	103 (40.9%)[b]	12 (40.0%)[a,b]	14 (56.0%)[b]

(c) Positive feedback

Baseline						
n	1645	81	1256	389	44	37
Continuous psychopathology (SDQ)						
emotional symptoms	1.9 ± 1.9	3.5 ± 2.9***	1.2 ± 1.3[a]	3.9 ± 2.1[b]	1.9 ± 1.9[c]	5.5 ± 2.6[d]
conduct problems	1.6 ± 1.6	3.0 ± 2.2***	1.5 ± 1.4[a]	2.1 ± 1.8[b]	2.3 ± 1.8[b]	3.8 ± 2.3[c]
hyperactivity	2.9 ± 2.2	4.7 ± 2.9***	2.6 ± 2.1[a]	3.6 ± 2.4[b]	3.6 ± 2.4[b]	6.1 ± 2.9[c]
impact	0.6 ± 1.3	2.2 ± 2.5***	0.4 ± 1.0[a]	1.2 ± 1.9[b]	0.9 ± 1.5[a,b]	3.7 ± 2.7[c]
Diagnostic categories						
any anxiety	389 (23.6%)	37 (45.7%)***	0	389 (100%)	0	37 (100%)
depression	51 (3.1%)	12 (14.8%)***	21 (1.7%)[a]	30 (7.7%)[b]	2 (4.5%)[a,b]	10 (27.0%)[c]
ODD	577 (35.1%)	51 (63.0%)***	357 (28.4%)[a]	220 (56.6%)[b]	25 (56.8%)[b]	26 (70.3%)[b]
2-year follow up						
n	1191	55	925	266	29	26
Male gender	561 (47.1%)	39 (70.9%)**	460 (49.7%)[a]	101 (38.0%)[b]	22 (75.9%)[c]	17 (65.4%)[a,c]
Continuous psychopathology (SDQ)						
emotional symptoms	1.7 ± 1.9	3.0 ± 3.0**	1.3 ± 1.6[a]	2.9 ± 2.3[b]	1.5 ± 2.2[a]	4.5 ± 3.0[c]
conduct problems	1.4 ± 1.4	2.1 ± 2.0*	1.3 ± 1.4[a]	1.7 ± 1.6[b]	1.7 ± 1.9[a,b,c]	2.6 ± 2.0[c]
hyperactivity	2.3 ± 2.0	3.8 ± 2.5***	2.2 ± 1.9[a]	2.7 ± 2.1[b]	2.9 ± 2.1[a,b]	4.9 ± 2.5[c]
impact	0.5 ± 1.4	1.3 ± 2.1**	0.4 ± 1.1[a]	1.0 ± 1.9[b]	1.0 ± 2.0[a,b]	1.7 ± 2.3[b]
Diagnostic categories						
any anxiety	235 (19.7%)	19 (34.5%)*	120 (13.0%)[a]	115 (43.2%)[b]	5 (17.2%)[a]	14 (53.8%)[b]
depression	43 (3.6%)	6 (10.9%)*	24 (2.6%)[a]	19 (7.1%)[b]	2 (6.9%)[a,b]	4 (15.4%)[b]
ODD	338 (28.4%)	25 (45.5%)**	230 (24.9%)[a]	108 (40.8%)[b]	10 (34.5%)[a,b]	15 (57.7%)[b]

*$p < .05$; **$p < .01$; ***$p < .001$.
a, b, c, d = different letters indicate a significant group difference from each other at $p<.05$ (Bonferroni corrected).

Table 4.3. Number of participants with ASD traits who met criteria for different DSM-IV defined anxiety diagnostic categories based on DAWBA computer predictions at probability band 2 or higher, at wave 1.

Diagnostic category	Anticipation (n=70)	Negative feedback (n=78)	Positive feedback (n=81)
Generalised anxiety	19	25	26
Social anxiety	13	17	17
Separation anxiety	10	10	11
Specific phobia	5	5	5
Agoraphobia	5	5	5
PTSD	4	3	4
OCD	3	3	3
Panic disorder	2	2	2
Number of different anxiety diagnoses			
0	40	43	44
1	14	15	16
2	6	10	11
3 or more	10	10	10

DAWBA, Development and Well-Being Assessment. *OCD*, obsessive-compulsive disorder. *PTSD*, post-traumatic stress disorder.

4.4.2 Behavioural performance

Paired-samples t-tests revealed that participants with high and low ASD traits both responded more accurately in "big win" compared to "no win" trials (Table 4.4), suggesting that both groups were motivated by the potential of winning a reward. The increase in response accuracy between "no win" and "big win" trials was higher for those with low vs. high ASD traits (Table 4.4).

Table 4.4. Percentage of accurate responses in "no win" vs. "big win" conditions in participants with low and high ASD traits.

	Low ASD traits	High ASD traits	**Steepness of accuracy increase** between no win and big win: low vs. high ASD traits
Reward anticipation	n = 1402	n = 70	
big win	67.4 ± 10.6	65.1 ± 9.2	
no win	53.8 ± 13.1	57.9 ± 10.4	
big win vs. no win	t=28.49, p<.001	t=3.90, p<.001	t=2.97, p=.003, d=0.39
Negative feedback	n = 1498	n = 76	
big win	67.1 ± 11.5	64.9 ± 9.4	

no win	52.1 ± 14.9	56.0 ± 12.0	
big win vs. no win	t=30.40, p<.001	t=4.68, p<.001	t=2.72, p=.007, d=0.34
Positive feedback	n = 1645	n = 81	
big win	66.9 ± 11.7	65.0 ± 9.6	
no win	51.7 ± 15.4	56.1 ± 12.1	
big win vs. no win	t=31.65, p<.001	t=4.73, p<.001	t=2.90, p=.004, d=0.35

4.4.3 Reward anticipation

We first conducted an ANOVA to test the effects of ASD traits and anxiety on brain activations during reward anticipation.

Main effects. We found a main effect of ASD traits severity and a main effect of anxiety (Table 4.5). Participants with high ASD traits (n=70) showed lower BOLD responses in the right superior frontal gyrus (SFG) extending to anterior and midcingulate regions relative to youth with low ASD traits (n=1402). Participants with anxiety symptoms displayed increased activation in the right middle frontal and middle temporal gyri (MFG and MTG), irrespective of ASD traits severity.

Table 4.5. The effects of ASD traits and anxiety on BOLD responses during reward anticipation.

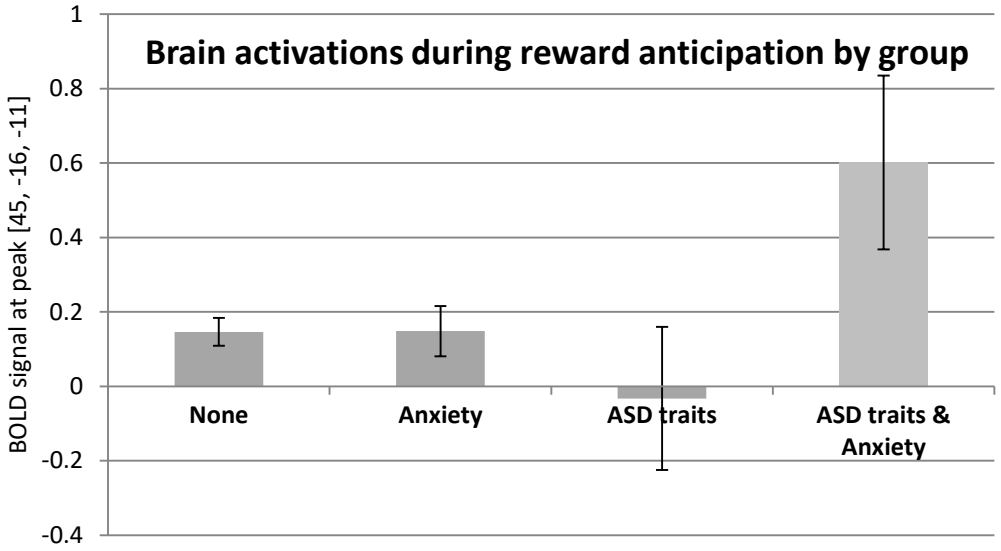
Region	BA	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	p (FWE)
Interaction: ASD traits x Anxiety							
R middle and superior temporal gyri, R insula	21/22/13	283	45 57 63	-16 -37 -16	-11 -8 -11	3.97 3.80 3.71	.001
High < Low ASD traits							
R superior and medial frontal gyri extending bilaterally to the dACC and MCC	9/24/32	936	-18 18 9	17 17 50	37 49 37	4.88 4.72 4.58	<.001
Any anxiety > No anxiety							
R middle frontal gyrus	8	254	39 21 42	23 59 8	43 37 49	4.41 3.89 3.66	.002
R middle temporal gyrus	21	209	45	-16	-11	3.98	.008

			60	-16	-5	3.67	
			57	-37	-11	3.47	
ASD_{ANX} > ASD_{ONLY}							
R middle temporal gyrus	21	257	57	-37	-8	4.56	<.001
			63	-13	-2	4.31	
			69	-37	-2	3.90	
R middle frontal gyrus	8	251	42	23	43	4.27	<.001
			42	11	52	3.71	
			42	32	43	3.45	
L insula, L inferior frontal gyrus	13/45/47	162	-42	17	-11	4.27	.011
			-36	17	1	3.43	
			-45	17	7	3.36	
L inferior parietal lobule	40	124	-57	-43	55	3.86	.047
			-48	-61	52	3.33	
			-66	-37	37	3.11	
L superior and middle temporal gyri	39/22/40	246	-60	-67	7	3.71	.001
			-51	-82	7	3.70	
			-51	-79	22	3.68	
R inferior parietal lobule	40	256	51	-52	55	3.60	<.001
			60	-34	55	3.49	
			48	-34	64	3.41	
ASD_{ANX} > ANX_{ONLY}							
L insula, L inferior frontal gyrus	47	186	-42	17	-8	4.68	.017
			-36	14	4	3.65	
			-54	20	1	3.57	
L and R cuneus and calcarine	18/17	222	-12	-82	10	4.30	.006
			12	-85	10	3.77	
			-6	-76	22	3.38	

ANX_{ONLY}, high anxiety & low ASD traits. *ASD_{ANX}*, high ASD traits & anxiety. *ASD_{ONLY}*, high ASD traits, low anxiety. *BA*, Brodmann area. *dACC*, dorsal anterior cingulate cortex. *FWE*, family-wise error correction. *L*, left hemisphere. *MCC*, middle cingulate cortex. *R*, right hemisphere.

Interaction. We also found an interaction between ASD traits and anxiety in a cluster encompassing right MTG, superior temporal gyrus and insula (Table 4.5). Figure 4.2 illustrates the interaction showing significantly increased activation at the cluster's peak in ASD_{ANX} relative to all other groups. Follow-up t-tests revealed that the ASD_{ANX} (n=30) group showed significantly increased brain activation relative to ASD_{ONLY} (n=40) in left insula and left inferior frontal gyrus (IFG), right MFG, as well as bilateral inferior parietal lobule (IPL) and temporal areas. ASD_{ANX} also displayed increased activation in left insula, left IFG, and posterior brain regions relative to ANX_{ONLY} (n=326).

Figure 4.2. Interaction between ASD traits and anxiety. Showing mean BOLD responses during reward anticipation and 95% confidence intervals at cluster peak [45, -16, -11] located in the middle temporal gyrus. Similar pattern of results emerged for a peak in the right insula.



4.4.3.1 Effects of ODD and depression

All results remained significant after controlling for the effects of ODD, and we found two additional clusters in the ASD-by-anxiety interaction (ASD_{ANX} > all other groups in left IFG, insula and MFG). After controlling for the effects of depression, all results remained significant, except for one cluster in the ASD_{ANX}>ASD_{ONLY} comparison (activations in left insula and left IFG no longer significant).

4.4.3.2 Emotional problems

Confirmatory analyses with the SDQ emotional problems subscale showed similarities to the anxiety analyses presented above (see Table 4.6). Consistent with anxiety findings, we found an interaction between ASD traits and emotional problems in the right insula and MTG, with ASD_{EMOT} showing the largest activation. We also found that youth with high levels of emotional problems showed increased activation in the right MFG, consistent with the main effect of anxiety found above. The main effect of ASD in frontal regions, found in anxiety analyses, did not reach significance (p_{FWE}=.144, k=109). However, the same areas were found in ASD_{EMOT} > $EMOT_{ONLY}$ and ASD_{ANX} > ANX_{ONLY} comparisons (left insula, left IFG, bilateral cuneus). Only one cluster (right MTG) remained significant for comparison ASD_{EMOT} > ASD_{ONLY} relative to ASD_{ANX} >

ASD_{ONLY}. Additionally, ASD_{EMOT} displayed increased activity in right thalamus and caudate relative to ASD_{ONLY}.

Table 4.6. The effects of ASD traits and emotional problems on BOLD responses during reward anticipation.

Region	BA	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)
Interaction: ASD traits x Emotional problems							
R thalamus, R caudate, R insula, R middle temporal gyrus	13	306	15	-13	10	4.30	<.001
			51	-40	-5	3.67	
			69	-25	-17	3.61	
R medial and superior frontal gyri/SMA, R midcingulate gyrus/dorsal ACC	6/8/32	237	9	-1	58	3.54	.002
			0	17	52	3.49	
			24	-16	58	3.33	
High > Low ASD traits							
L insula extending to L inferior frontal gyrus	13/47	249	-33	35	13	4.07	.002
			-36	14	1	3.97	
			-42	47	1	3.34	
L posterior cingulate gyrus, L cuneus	31/18	252	-6	-67	13	3.77	.001
			-9	-82	13	3.65	
			-15	-79	22	3.43	
High > Low emotional problems							
R middle and inferior frontal gyri	47	141	45	26	-14	4.02	.047
			39	41	-11	3.48	
			51	-40	-5	3.37	
ASD_{EMOT} > ASD_{ONLY}							
R thalamus, R caudate		138	12	-13	10	3.64	.022
			27	-31	16	3.47	
			27	-22	1	3.31	
R middle temporal gyrus	21	131	54	-16	-17	3.49	.030
			60	-55	-2	3.30	
			72	-28	-17	3.21	
ASD_{EMOT} > $EMOT_{ONLY}$							
L insula extending to L inferior frontal gyrus	13/47	201	-36	17	1	4.47	.003
			-42	17	-5	4.10	
			-45	2	1	3.71	

L and R lingual gyrus, L and R cuneus extending to the posterior cingulate	18/17	344	-6 6 -27	-67 -76 -73	13 -5 4	3.76 3.66 3.57	<.001

ACC, anterior cingulate cortex. ASD_{EMOT}, high ASD traits & emotional problems. ASD_{ONLY}, high ASD traits, low emotional problems. *BA*, Brodmann area. $EMOT_{ONLY}$, high emotional problems & low ASD traits. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere. *SMA*, supplementary motor area.

4.4.3.3 Effects of different anxiety categories

Next, we tested whether reward anticipation processing in youth with high ASD traits was associated with the number of anxiety disorders, or with specific types of anxiety disorders. We found a significant positive correlation between the number of anxiety disorders and brain activation in right MFG (Table 4.7). Since only three participants without GAD met criteria for social anxiety, we compared participants with GAD with (n=10) vs. without (n=9) co-occurring social anxiety symptoms. As shown in Table 4.8, participants with combined GAD and social anxiety symptoms had significantly decreased brain activation in bilateral orbitofrontal regions compared to those with GAD without social anxiety.

Table 4.7. Correlation between the number of anxiety disorders and brain activation during reward anticipation in 70 young people with high ASD traits.

Region	**BA**	**Cluster size (voxels)**	peak MNI coordinates **x**	**y**	**z**	**Z**	***p* (FWE)**
Positive correlation							
R middle frontal gyrus	8/9	159	45 42 42	23 32 11	46 43 52	4.52 3.59 3.49	.011

BA, Brodmann area. *FWE*, family-wise error correction. *R*, right hemisphere.

Table 4.8. Regions of differential activation during reward anticipation in young people with high ASD traits and co-occurring generalised anxiety disorder (GAD; n=9) vs. co-occurring GAD and social anxiety (n=10) [6].

Region	BA	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)
GAD > GAD and Social Anxiety							
L and R rectal gyrus, L and R medial frontal gyrus	11/25/32	262	3	44	-17	4.22	<.001
			9	35	-5	4.03	
			-12	38	-5	3.73	

BA, Brodmann area. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere.

4.4.4 Negative feedback

Main effects. We found a main effect of ASD traits severity and a main effect of anxiety (Table 4.9). Participants with high ASD traits (n=78) showed lower activation in right superior and medial frontal gyri relative to youth with low ASD traits (n=1523). Irrespective of ASD traits severity, participants with anxiety symptoms (n=396) showed decreased activation in the following regions following negative feedback: bilateral caudate, bilateral IFG, right SFG, left MFG, left IPL, and left MTG. The results are summarised against reward anticipation in Figure 4.3.

Table 4.9. The effects of ASD traits and anxiety on BOLD responses during negative reward feedback.

Region	BA	Cluster size (voxels)	peak MNI coordinates x	Y	z	Z	*p* (FWE)
Interaction: ASD traits x Anxiety							
R superior and medial frontal gyri	10	223	21	59	7	4.95	.003
			27	59	1	4.84	
			18	47	22	4.34	

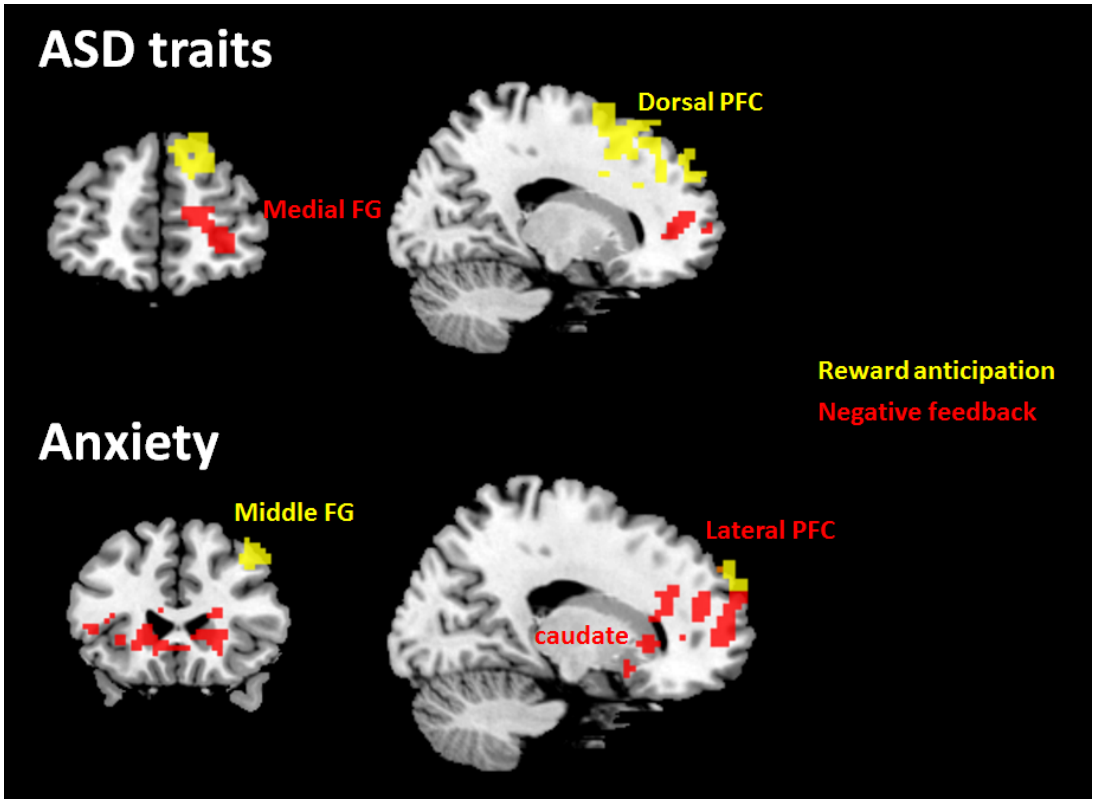
[6] NB. Participants in the "GAD" group also met criteria for separation anxiety (n=3), agoraphobia (n=1), and depression (n=3). Participants in the "GAD and social anxiety" group met criteria for separation anxiety (n=3), specific phobia (n=3), PTSD (n=3), OCD (n=2), panic disorder (n=1), agoraphobia (n=1), and depression (n=4).

R caudate, R putamen, R middle and inferior frontal gyri	10	301	21 27 21	23 38 23	1 -5 13	4.73 4.46 3.76	<.001
L middle temporal gyrus		183	-51 -57 -24	-76 -70 -85	13 10 16	4.05 3.87 3.36	.010
High < Low ASD traits							
R superior and medial frontal gyri	10	137	21 18 30	44 53 56	1 13 -2	4.33 4.06 3.71	.046
Any anxiety < No anxiety							
R superior frontal gyrus extending to R medial frontal gyrus	10	145	21 21 6	59 62 65	7 19 28	5.08 3.62 2.75	.034
R caudate, R inferior frontal gyrus		449	18 21 27	47 23 38	22 1 -5	4.79 4.67 4.29	<.001
L inferior parietal lobule		184	-36 -24 -24	-46 -58 -52	28 25 34	4.62 4.53 3.87	.009
L middle temporal gyrus		182	-60 -51 -51	-70 -76 -67	7 13 13	4.12 3.85 3.33	.010
L middle and inferior frontal gyrus		193	-48 -30 -30	29 44 41	-5 22 14	4.04 3.97 3.72	.007
L caudate extending to the subgenual ACC	24/25	144	-9 -18 -12	23 23 14	-5 4 4	3.71 3.67 3.41	.036
ASD_{ANX} < ASD_{ONLY}							
R middle and inferior frontal gyri (extending to the midcingulate/ACC)	46/32	143	27 33 42	32 38 35	31 16 13	4.30 4.12 3.66	.015
L inferior and middle frontal gyri	46	136	-48 -36 -45	29 26 32	-2 13 19	4.20 3.39 3.38	.020
L rolandic operculum / precentral gyrus, L inferior frontal gyrus	22	120	-63 -54 -57	5 2 14	4 7 7	4.00 3.65 3.55	.040
		179	9	-10	22	3.60	0.004

L and R lateral ventricles, corpus callosum (extending to L caudate)			-6	-19	25	3.35	
			-15	-1	25	3.35	
ASD_{ANX} < ANX_{ONLY}							
R putamen and R caudate extending to subcallosal gyrus / gyrus rectus, R superior and medial frontal gyri	34/13/10	472	27 15 18	59 56 20	1 7 1	5.33 4.70 3.89	<.001
L putamen and L caudate, L middle frontal gyrus	47	227	-30 -12 -21	44 11 23	-8 1 1	4.17 4.00 3.88	.003

ACC, anterior cingulate cortex. ANX_{ONLY}, high anxiety & low ASD traits. ASD_{ANX}, high ASD traits & anxiety. ASD_{ONLY}, high ASD traits, low anxiety. *BA,* Brodmann area. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere.

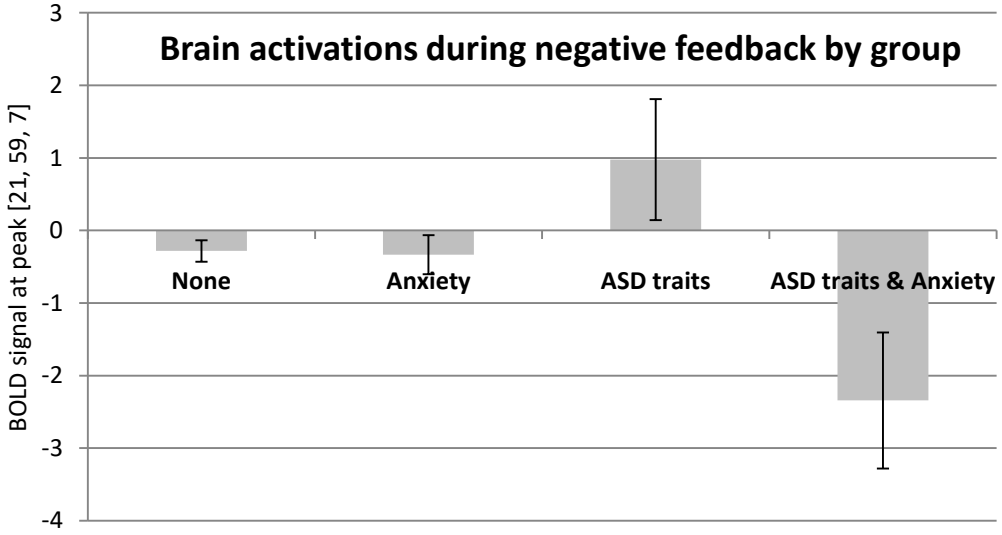
Figure 4.3. Comparative summary of the main effects of ASD traits and anxiety on BOLD responses during reward anticipation (yellow) and negative feedback (red). Showing whole-brain-level ANOVA results (FWE corrected) overlaid on a T1-weighted structural brain image. For ASD traits, the areas shown were less activated in those with high vs. low ASD traits. For anxiety, the areas shown were more activated during anticipation, but less activated during negative feedback, in those with high vs. low anxiety.



FG, frontal gyrus. *PFC*, prefrontal cortex.

Interaction. We also found an interaction between ASD traits and anxiety severity in right caudate and putamen, prefrontal regions, and left MTG. While anxiety did not affect brain activations in these regions in youth with low ASD traits, young people with ASD traits and anxiety showed markedly lower activations compared to ASD_{ONLY} (Figure 4.4). Follow-up t-tests revealed that ASD_{ANX} (n=35) had significantly decreased brain activation in bilateral MFG and IFG extending to the anterior cingulate, left precentral gyrus, and corpus callosum extending to the left caudate compared to ASD_{ONLY} (n=43). ASD_{ANX} also displayed decreased activation in bilateral caudate and putamen relative to ANX_{ONLY} (n=361).

Figure 4.4. Interaction between ASD traits and anxiety. Showing mean BOLD responses during negative reward feedback and 95% confidence intervals at cluster 1 peak [21, 59, 7] in right superior frontal gyrus. The same pattern of results emerged for the two other clusters with peaks in right caudate and left middle temporal gyrus, and middle frontal gyrus.



4.4.4.1 Effects of ODD and depression

After controlling for the effects of ODD, the ASD-by-anxiety interaction remained significant, and we found an additional cluster encompassing the left caudate and left IFG. The main effect of anxiety remained significant in three out of six previously-found clusters (the following clusters lost significance after controlling for ODD: right SFG k=120, p_{FWE}=.084; left MFG and IFG k=114, p_{FWE}=.104; left caudate and sgACC k=121, p_{FWE}=.081). The main effect of ASD was just below threshold for significance (frontal cluster k=131, p_{FWE}=.056). The ASD_{ANX}< ANX_{ONLY} t-test remained significant, but 3/4 clusters in the ASD_{ANX}<ASD_{ONLY} comparison lost significance (right MFG and IFG k=105, p_{FWE}=.084; left MFG and IFG k=109, p_{FWE}=.070; left IFG/rolandic operculum k=79, p_{FWE}=.260).

All results remained significant after controlling for the effects of depression, except for one cluster in the ASD_{ANX}<ASD_{ONLY} comparison (activation in left rolandic operculum no longer significant, k=107, p_{FWE}=.084).We also found two additional clusters encompassing left IFG, left caudate and left putamen in the ASD-by-anxiety interaction.

4.4.4.2 Irritability symptoms

We then tested whether neural activation patterns following negative feedback were associated with symptoms of irritability. However, no significant clusters were found in regressions within either the low or the high ASD traits groups.

4.4.4.3 Emotional problems

Confirmatory analyses with SDQ emotional problems subscale (Table 4.10) brought similar results to our anxiety analyses. Consistent with anxiety findings, we found an interaction between ASD traits and emotional problems in right MFG and IFG. We also found the main effect of ASD traits in right medial and superior frontal gyri. Youth with high levels of emotional problems showed decreased activation in frontal regions (bilateral IFG, MFG, SFG), consistent with the main effect of anxiety. Some of the same areas were found in ASD_{EMOT} < ASD_{ONLY} and ASD_{ANX} < ASD_{ONLY} comparisons (bilateral IFG, right MFG, left precentral gyrus); and ASD_{EMOT} < TD_{EMOT} and ASD_{ANX} < TD_{ANX} (right caudate, right medial and superior frontal gyri).

Table 4.10. The effects of ASD traits and emotional problems on BOLD responses patterns following negative feedback.

Region	BA	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)
Interaction: ASD traits x Emotional problems							
R inferior and middle frontal gyri, R dorsal anterior cingulate gyrus	10/32	314	39	35	10	4.07	.002
			18	26	13	3.96	
			48	38	10	3.94	
High < Low ASD traits							
R medial and superior frontal gyri	10	200	21	44	1	4.50	.024
			18	53	10	4.34	
			30	56	-2	4.23	

High < Low emotional problems							
L midcingulate gyrus, corpus callosum	24	198	-15 -6 -15	5 -7 -10	31 28 28	4.67 3.91 3.48	.025
L and R inferior, middle and superior frontal gyri (extending to dACC), R insula, R putamen, R precentral and superior temporal gyri	9/10/46/45/44/13	2074	27 63 21	32 -25 53	31 19 1	4.55 4.51 4.49	<.001
ASD_{EMOT} < ASD_{ONLY}							
R inferior frontal and precentral gyri, sub-gyral (R frontal lobe)		950	42 30 42	35 38 -16	10 19 37	4.70 4.35 4.03	<.001
L paracentral lobule, corpus callosum, L and R supplementary motor area	6	626	0 -15 -3	-19 5 -4	70 28 64	4.17 3.91 3.68	<.001
L inferior and middle frontal gyri	10/46	295	-48 -45 -27	50 44 68	1 25 25	3.95 3.49 3.44	<.001
L precentral, inferior frontal, and superior temporal gyri	22/9	201	-60 -60 -48	2 8 2	4 28 28	4.17 3.71 3.61	.007
ASD_{EMOT} < $EMOT_{ONLY}$							
R caudate, R medial and superior frontal gyri extending to the anterior cingulate	10	317	15 27 15	59 59 50	7 -2 1	4.75 4.26 3.86	<.001

ASD_{EMOT}, high ASD traits & emotional problems. *ASD_{ONLY}*, high ASD traits, low emotional problems. *BA*, Brodmann area. *dACC*, dorsal anterior cingulate cortex. *$EMOT_{ONLY}$*, high emotional problems & low ASD traits. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere.

4.4.4.4 Effects of different anxiety categories

We found a significant negative correlation between the number of anxiety disorders and brain activation in right MFG and IFG, bilateral medial frontal gyrus and paracentral lobules, and right supplementary motor area (SMA), precuneus, and supramarginal gyrus (Table 4.11). Moreover, participants with combined GAD and social anxiety symptoms (n=14) had significantly decreased brain activation in six clusters compared to those with GAD without social anxiety (n=10). Regions of relatively decreased activation included bilateral middle, medial, and superior frontal gyri, bilateral SMA and the cerebellum (Table 4.12).

Table 4.11. Correlation between the number of anxiety disorders and brain activation in response negative reward feedback in 78 young people with high ASD traits.

Region	BA	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)
Negative correlation							
R middle and inferior frontal gyri	46	229	42	35	13	4.48	.001
			27	32	31	4.35	
			33	38	16	4.30	
R inferior frontal and precentral gyri (extending to the R insula)	43, 44	215	45	2	10	3.99	.001
			54	-10	10	3.58	
			54	17	7	3.17	
R supramarginal gyrus	40	172	54	-40	31	3.95	.006
			60	-49	25	3.35	
			63	-31	43	3.32	
R precuneus, sub-gyral areas of the parietal lobe		126	12	-58	49	3.71	0.036
			18	-49	64	3.41	
			21	-49	40	3.30	
L and R medial frontal gyrus, R SMA/superior frontal gyrus, L and R paracentral lobule	6	382	-9	-25	64	3.67	<.001
			12	-7	64	3.67	
			0	-19	70	3.67	

BA, Brodmann area. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere, *SMA*, supplementary motor area.

Table 4.12. Regions of differential activation to negative reward feedback in young people with high ASD traits and co-occurring generalised anxiety disorder (GAD; n=11) vs. co-occurring GAD and social anxiety (n=14)[7].

Region	BA	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)
GAD > GAD and Social Anxiety							
		2846	57	-55	28	4.83	<.001

[7] NB. Participants in the "GAD" group also met criteria for separation anxiety (n=3), agoraphobia (n=1), and depression (n=4). Participants in the "GAD and social anxiety" group met criteria for separation anxiety (n=3), specific phobia (n=3), PTSD (n=3), OCD (n=2), panic disorder (n=1), agoraphobia (n=1), and depression (n=5).

R middle occipital and temporal gyri, R supramarginal gyrus, R middle and inferior frontal gyri, L and R cuneus and precuneus, L midcingulate gyrus, L and R SMA, medial and superior frontal gyri	40, 5, 9, 24, 6		48	-73	-5	4.79	
			63	-40	22	4.37	
L superior frontal gyrus	10	103	-21	53	16	4.47	.050
			-24	59	22	4.37	
			-18	56	7	3.50	
L middle frontal gyrus, L and R superior frontal gyrus	8	320	-45	17	43	4.46	<.001
			-21	29	52	3.85	
			6	26	55	3.84	
L and R cerebellum (extending to R lingual gyrus and L middle occipital gyrus)		1051	-3	-70	-35	4.14	<.001
			18	-70	-26	3.83	
			-24	-64	-26	3.80	
R fusiform gyrus and cerebellum		110	39	-55	-20	3.78	.036
			27	-43	-23	3.52	
			51	-61	-26	3.22	
L postcentral/supramarginal gyrus	40	206	-63	-25	13	3.65	.001
			-63	-37	25	3.55	
			-66	2	13	3.41	

BA, Brodmann area. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere.

4.4.5 Positive feedback

Main effects. Across the whole sample, we found a main effect of ASD traits severity. Youth with high ASD traits (n=81) displayed increased activation in bilateral thalamus and pallidum, as well as right putamen, compared to youth with low ASD traits (n=1645; Table 4.13). This finding remained significant after controlling for the effects of ODD, but lost significance after controlling for the effects of depression (right-sided cluster p_{FWE}=.061, k=148; left-sided cluster p_{FWE}=.671, k=65). No main effect of anxiety was found.

Table 4.13. The effects of ASD traits and anxiety on brain activation patterns following positive feedback.

Region	BA	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)
High > Low ASD traits							
R thalamus, R putamen, R globus pallidus		163	30	-4	4	5.03	.039
			24	-10	4	4.78	

		21	-22	7	3.74	
L thalamus, L globus pallidus, midbrain, extending to L hippocampus	185	15 -15 -9	-16 -7 22	-5 1 1	4.23 4.21 3.66	.021

BA, Brodmann area. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere.

Interaction. We did not find a significant interaction.

4.4.5.1 Emotional problems

We did not find a main effect of ASD traits in our analyses with SDQ emotional subscale. Instead, we found a main effect of emotional problems in the left middle occipital gyrus (Table 4.14). No interaction was found.

Table 4.14. The effects of ASD traits and emotional problems on brain activation patterns following positive feedback.

Region	BA	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)
High < Low emotional problems							
L middle occipital gyrus		191	-51 -48 -30	-70 -82 -70	-8 7 10	4.10 3.68 3.41	.030

BA, Brodmann area. *FWE*, family-wise error correction. *L*, left hemisphere.

4.4.6 Longitudinal predictions

We investigated whether brain correlates of reward processing found in our interaction analyses, and relevant to the comorbid group, represent a mechanism underlying successive comorbidity between ASD traits and anxiety.

4.4.6.1 Reward anticipation

The following ROI predictors were tested (all right-sided): MTG, insula, BA 32 (dorsal cingulate), caudate, thalamus, and medial frontal gyrus (medFG, defined using the 'frontal_sup_medial' mask from the automatic anatomical labelling atlas in WFUPickatlas).

High ASD traits. In participants with high ASD traits, increased activations in the right medFG and right BA 32 during reward anticipation were associated with a higher likelihood of anxiety at follow-up, with baseline anxiety and ASD traits included in the model ($OR_{medFGright}$=62.33, 95% CI [1.46-2668.54], *p*=.031; $OR_{BA32right}$=33.22, 95% CI [1.47-750.36], *p*=.028; Table 4.15); however note wide confidence intervals.

Low ASD traits. None of the ROIs significantly predicted new-onset anxiety in participants with low ASD traits.

Interaction effects across the whole sample. The interaction term between ASD traits and right medFG was a significant predictor of new-onset anxiety at follow-up (OR=17.34, 95% CI [1.45-207.03], *p*=.024). Likewise, the interaction between ASD traits and right BA 32 significantly predicted future anxiety (OR=15.75, 95% CI [1.32-187.48], *p*=.029).

No significant results were found for the remaining ROIs.

Table 4.15. Predicting anxiety status (no/yes) at two-year follow-up by right medial frontal gyrus (medFG) and right Brodmann area 32 (BA32) activations during reward anticipation at baseline. Presented separately for participants with high and low ASD traits.

	ROI: right medFG		**ROI: right BA 32**	
Variable	*Odds Ratio (95% Confidence Interval)*			
	High ASD traits	Low ASD traits	High ASD traits	Low ASD traits
ROI	62.33* (1.46-2668.54)	0.93 (0.63-1.39)	33.22* (1.47-750.36)	0.84 (0.55-1.28)
female gender	12.10* (1.37-107.13)	1.84** (1.29-2.61)	7.81* (1.07-56.91)	1.83** (1.28-2.60)
handedness	1.06 (0.13-8.82)	1.49 (0.86-2.58)	0.96 (0.13-7.34)	1.48 (0.85-2.57)
site	1.09 (0.76-1.58)	1.03 (0.96-1.11)	1.10 (0.78-1.55)	1.03 (0.96-1.11)
WISC Verbal Comprehension	1.09 (1.00-1.18)	1.00 (0.98-1.01)	1.07 (1.00-1.15)	1.00 (0.98-1.01)
WISC Reasoning	1.00 (0.94-1.06)	0.99 (0.98-1.00)	1.01 (0.95-1.07)	0.99 (0.98-1.00)
Baseline ASD traits	1.13 (0.96-1.33)	1.02 (0.93-1.12)	1.13 (0.96-1.33)	1.02 (0.93-1.12)
Baseline anxiety ("yes")	16.29* (1.70-155.60)	5.50*** (3.89-7.78)	12.15* (1.51-97.98)	5.49*** (3.88-7.76)
LR X^2	23.36**	121.97***	23.04**	122.49***
Pseudo R^2	0.36	0.12	0.36	0.12
Log likelihood	-20.37	-437.71	-20.53	-437.45

*p < .05; **p < .01; ***p<.001. *ROI*, region-of-interest.

4.4.6.2 Negative feedback

None of the ROIs we tested (right-sided: medFG, MFG, caudate, putamen, BA 32; left MTG) predicted dichotomous anxiety status at follow-up in either ASD traits group.

4.4.7 Analyses with the core ASD group

As a plausibility check, we investigated the effects of anxiety and emotional problems on reward processing in participants with more strictly defined ASD symptoms, to ensure that our main results were not simply accounted for by the threshold used to define the high ASD traits group. The core ASD group included participants who belonged to band 2 or above based on the DAWBA ASD algorithm. All but two participants in the core ASD group also belonged to the group with high ASD traits in our main analyses, with the two additional participants not meeting our inclusion criteria due to only endorsing two and not three items on the repetitive behaviours DAWBA ASD subscale.

Table 4.16 shows participant characteristics for the core ASD group ("High" ASD traits) compared to participants with low ASD traits, and "Medium" ASD traits, i.e. those who displayed high ASD traits based on the definition used in our main analyses but were placed in bands 0 or 1 according to the DAWBA ASD algorithm. As shown, the core ASD group was the most impaired on all measures, except for IQ which did not differ significantly between the low and core ASD groups.

Table 4.16. Participant characteristics across three levels of ASD traits severity.

	ASD traits		
	Low	**Medium**	**High ("Core ASD")**
(a) Reward anticipation			
n	1400	45	27
Male gender	656 (46.9%)[a]	29 (64.4%)[b]	19 (70.4%)[b]
Age in years	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4
WISC Verbal	111.9 ± 14.7[a]	105.6 ± 14.3[b]	113.4 ± 15.5[a]
WISC Reasoning	108.1 ± 13.9	109.4 ± 12.7	108.0 ± 16.7
ASD symptoms (DAWBA)			
total	0.3 ± 1.5[a]	15.4 ± 4.5[b]	20.4 ± 5.5[c]
social difficulties	0.2 ± 1.1[a]	8.9 ± 3.4[b]	11.8 ± 3.4[c]
repetitive behaviours	0.1 ± 0.5[a]	5.8 ± 2.5[b]	8.2 ± 4.8[c]
language development	0.1 ± 0.3[a]	0.7 ± 0.9[b]	0.4 ± 0.8[c]
Social Aptitudes Scale	24.8 ± 5.5[a]	20.2 ± 6.7[b]	13.7 ± 6.7[c]
Continuous psychopathology (SDQ)			
emotional symptoms	1.8 ± 1.9[a]	3.0 ± 2.6[b]	4.2 ± 3.2[c]
conduct problems	1.6 ± 1.5[a]	2.3 ± 2.0[b]	4.0 ± 2.2[c]
hyperactivity	2.8 ± 2.2[a]	3.7 ± 2.7[b]	6.0 ± 2.8[c]
impact	0.6 ± 1.3[a]	1.6 ± 2.5[b]	3.3 ± 2.2[c]
Diagnostic categories			
any anxiety	324 (23.1%)[a]	14 (31.1%)[a]	18 (66.7%)[b]
depression	43 (3.1%)[a]	6 (13.3%)[b]	5 (18.5%)[c]
ODD	481 (34.4%)[a]	24 (53.3%)[b]	21 (77.8%)[c]
(b) Negative feedback			
n	1521	50	30
Male gender	737 (48.5%)[a]	34 (68.0%)[b]	21 (70.0%)[b]
Age in years	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4
WISC Verbal	111.2 ± 14.7[a]	104.7 ± 15.2[b]	111.9 ± 16.3[a]
WISC Reasoning	107.7 ± 14.2	107.1 ± 2.0	107.5 ± 2.6
ASD symptoms (DAWBA)			
total	0.3 ± 1.5[a]	15.0 ± 4.3[b]	20.3 ± 5.6[c]
social difficulties	0.2 ± 1.1[a]	8.7 ± 3.5[b]	11.9 ± 4.4[c]
repetitive behaviours	0.1 ± 0.6[a]	5.6 ± 2.5[b]	8.0 ± 4.6[c]
language development	0.1 ± 0.3[a]	0.7 ± 1.0[b]	0.4 ± 0.9[c]
Social Aptitudes Scale	24.8 ± 5.6[a]	20.1 ± 6.6[b]	14.9 ± 7.5[c]
Continuous psychopathology (SDQ)			
emotional symptoms	1.9 ± 1.9[a]	3.0 ± 2.6[b]	4.4 ± 3.2[c]
conduct problems	1.6 ± 1.5[a]	2.4 ± 2.0[b]	3.8 ± 2.1[c]
hyperactivity	2.8 ± 2.2[a]	3.9 ± 2.6[b]	5.9 ± 2.8[c]
impact	0.6 ± 1.3[a]	1.5 ± 2.3[b]	3.2 ± 2.2[c]
Irritability symptoms (DAWBA)	0.5 ± 1.1[a]	1.3 ± 1.7[b]	2.4 ± 1.7[c]
Diagnostic categories			
any anxiety	359 (23.6%)[a]	16 (32.0%)[a]	21 (70.0%)[b]

depression	47 (3.1%)[a]	8 (16.0%)[b]	6 (20.0%)[b]
ODD	532 (35.0%)[a]	28 (56.0%)[b]	23 (76.7%)[b]

(c) Positive feedback	**Low**	**Medium**	**High**
n	1643	52	31
Male gender	794 (48.3%)[a]	36 (69.2%)[b]	22 (71.0%)[b]
Age in years	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4
WISC Verbal	111.3 ± 14.8[a]	105.2 ± 14.7[b]	111.5 ± 16.2[a]
WISC Reasoning	107.5 ± 14.1	107.9 ± 13.6	107.0 ± 16.7
ASD symptoms (DAWBA)			
total	0.3 ± 1.5[a]	15.5 ± 4.2[b]	20.3 ± 5.5[c]
social difficulties	0.2 ± 1.1[a]	8.9 ± 3.4[b]	11.7 ± 4.5[c]
repetitive behaviours	0.1 ± 0.5[a]	5.9 ± 2.6[b]	8.1 ± 4.5[c]
language development	0.1 ± 0.3[a]	0.7 ± 0.9[b]	0.5 ± 1.0[c]
Social Aptitudes Scale	24.7 ± 5.5[a]	19.5 ± 6.7[b]	14.9 ± 7.4[c]
Continuous psychopathology (SDQ)			
emotional symptoms	1.9 ± 1.9[a]	3.0 ± 2.5[b]	4.3 ± 3.2[c]
conduct problems	1.6 ± 1.6[a]	2.5 ± 2.1[b]	3.8 ± 2.1[c]
hyperactivity	2.9 ± 2.2[a]	4.0 ± 2.7[b]	5.9 ± 2.8[c]
impact	0.6 ± 1.3[a]	1.7 ± 2.6[b]	3.1 ± 2.2[c]
Diagnostic categories			
any anxiety	387 (23.6%)[a]	18 (34.6%)[a]	21 (67.7%)[b]
depression	50 (3.0%)[a]	7 (13.5%)[b]	6 (19.4%)[b]
ODD	575 (35.0%)[a]	29 (55.8%)[b]	24 (77.4%)[b]

a, b, c, d = different letters indicate a significant group difference from each other at p<.05 (Bonferroni corrected)

We then conducted t-tests within the core ASD traits group to investigate whether the effects of anxiety and emotional problems on reward processing found in our main analyses hold in participants who met more strict ASD criteria.

4.4.7.1 Reward anticipation

No differences in the neural correlates of reward processing were found between participants with ASD symptoms with vs. without co-occurring anxiety.

Effects of ODD and depression. After depression (but not ODD) was added as a covariate, significant clusters emerged whereby ASD_{ANX} showed a higher activation in right-sided MTG[8] and MFG relative to ASD_{ONLY}, as found in the analyses with less strictly defined ASD traits above. An additional cluster in right precuneus was also found (Table 4.17 a).

[8] p_{FWE}=.068 for this cluster when depression not added as a covariate into the model.

Emotional problems. Six significant clusters were found in the ASD_{EMOT} > ASD_{ONLY} contrast (Table 4.18 a). Similarly to the main analyses with ASD traits above, the ASD_{EMOT} group showed increased activation in the right thalamus and temporal regions, as well as right insula and midcingulate regions that emerged in the ASD-by-emotional problems interaction. Additional clusters in the posterior brain regions emerged (left cerebellum, bilateral posterior cingulate).

4.4.7.2 Negative feedback

We found increased activation in left parahippocampal and fusiform gyri in the ASD_{ANX} > ASD_{ONLY} contrast (Table 4.17 b), not found in our main analyses with the less strictly defined ASD traits above.

Effects of ODD and depression. The finding remained significant after adding ODD, and depression, as additional covariates.

Irritability symptoms. Similarly to our main analyses, no association was found between irritability symptoms and brain activation patterns following negative feedback.

Emotional problems. Nine significant clusters were found in the ASD_{EMOT} < ASD_{ONLY} contrast (Table 4.18 b), including areas that were found in the same contrast with less strictly defined ASD traits (L MFG, SMA, R precentral gyrus) and in the ASD-by-emotional problems interaction (R IFG, R MFG). Additional clusters were found in the frontal regions (right medFG, bilateral subgenual ACC) and posterior regions (bilateral precuneus, occipital gyri).

4.4.7.3 Positive feedback

A single significant cluster was found in the temporal regions in the ASD_{ANX} < ASD_{ONLY} contrast (Table 4.17 c), not found in our main analyses above. The effect remained significant after ODD, and depression, were added to the model. No results were found using the emotional problems variable.

Table 4.17. The effects of anxiety on brain activation patterns during reward processing in participants with ASD traits defined using the algorithm in the DAWBA ASD section (ASD band 2 and above). For reward anticipation condition, the significant clusters shown only emerged after adding depression to the model as an additional covariate.

Region	BA	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)
(a) REWARD ANTICIPATION showing results after adding depression as an additional covariate							
ASD_{ANX} > ASD_{ONLY} (n=18 vs. n=9)							
R middle and superior temporal gyri	37/39/22	113	51	-67	1	4.01	.033
			51	-58	13	3.69	
			51	-46	7	3.09	
R middle frontal and precentral gyri	8/9	136	51	23	46	3.75	.011
			33	23	46	3.53	
			57	8	40	3.40	
R precuneus	7	115	3	-64	64	3.66	.030
			-3	-76	61	3.61	
			27	-67	61	3.41	
(b) NEGATIVE FEEDBACK							
ASD_{ANX} > ASD_{ONLY} (n=21 vs. n=9)							
L parahippocampal and fusiform gyri	19/28	137	-33	-49	-8	3.97	.012
			-18	-31	-11	3.63	
			-48	-49	-8	3.26	
(c) POSITIVE FEEDBACK							
ASD_{ANX} < ASD_{ONLY} (n=21 vs. n=10)							
L middle and superior temporal gyri, supramarginal gyrus		200	-27	-52	28	4.20	.001
			-39	-49	25	3.65	
			-54	-55	19	3.33	

BA, Brodmann area. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere.

Table 4.18. The effects of emotional problems on brain activation patterns during reward processing in participants with ASD traits defined using the algorithm in the DAWBA ASD section (ASD band 2 and above).

Region	BA	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)
(a) REWARD ANTICIPATION							
ASD_{EMOT} > ASD_{ONLY} (n=9 vs. n=18)							
R paracentral lobule extending to the midcingulate gyrus	5	307	6	-34	52	4.83	<.001
			12	-40	64	3.62	
			33	-22	52	3.56	
L and R posterior cingulate	23/31	557	-54	-37	31	4.76	<.001
			-18	-73	16	3.98	
			3	-37	10	3.88	
R thalamus, R insula, R inferior parietal lobule / supramarginal gyrus	13/40	349	15	-25	13	4.52	<.001
			57	-55	31	3.98	
			30	-31	19	3.59	
L precentral, postcentral and superior temporal gyri		189	-60	-25	22	4.49	.001
			-36	-22	43	4.05	
			-63	-13	31	3.63	
L precentral gyrus extending to L middle frontal gyrus		122	-30	2	25	3.80	.016
			-45	-1	28	3.59	
			-51	26	43	3.32	
L cerebellum		162	-30	-85	-32	3.67	.002
			-33	-67	-35	3.62	
			-27	-85	-17	3.60	
(b) NEGATIVE FEEDBACK							
ASD_{EMOT} < ASD_{ONLY} (n=14 vs. n=16)							
L and R precuneus	7	310	-12	-76	46	4.82	<.001
			-6	-64	55	3.65	
			0	-46	58	3.60	
L middle and superior frontal gyri extending to the anterior cingulate	10/46/9	649	-42	53	13	4.59	<.001
			-33	53	28	4.19	
			3	17	28	4.08	
L inferior occipital, lingual and fusiform gyri	18	295	-18	-94	-17	4.53	<.001
			-15	-94	-17	4.41	
			-54	-49	-32	3.93	

L inferior parietal lobule	40	176	-57	-40	37	4.44	.002
			-66	-25	25	3.83	
			-54	-31	34	3.65	
R middle occipital gyrus	19	148	39	-85	4	3.89	.006
			18	-103	-11	3.38	
			21	-88	7	3.35	
R middle, superior and medial frontal gyri	10/9	371	42	53	16	3.80	<.001
			27	68	16	3.71	
			36	65	13	3.67	
L and R superior frontal gyrus/SMA	6	148	12	2	67	3.50	.006
			-6	-7	76	3.49	
			3	8	61	3.49	
L and R subgenual ACC / subcallosal gyrus	25	115	-3	23	-11	3.35	.029
			12	23	-20	3.25	
			6	11	-20	3.12	
R inferior frontal and precentral gyri		167	63	-1	16	3.18	.003
			57	29	10	3.15	
			48	14	7	3.11	

ACC, anterior cingulate cortex. *BA,* Brodmann area. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere. *SMA*, supplementary motor area.

4.5 Discussion

We found independent effects of ASD traits and anxiety on neural correlates of reward processing. We also found interaction effects whereby youth with combined ASD traits and anxiety showed distinctively high right MTG and insula activation when anticipating reward, and low prefrontal activation during negative feedback. Moreover, in participants with high ASD traits, brain activation patterns during reward anticipation predicted new onset of anxiety two years later.

ASD traits. During both reward anticipation and negative feedback, we observed attenuated BOLD activation in prefrontal regions in participants with high compared to low ASD traits. When anticipating reward, participants with high ASD traits showed reduced activation in dorsal ACC and right dorsal PFC (BA 9), regions involved in working memory, cognitive salience detection, and monitoring of reward-based behavioural responses (Richards et al., 2013; Ridderinkhof, van den Wildenberg, Segalowitz, & Carter, 2004; Wallis & Miller, 2003). This effect remained significant after controlling for the effects of depression and ODD symptoms. Participants with ASD traits may attach less salience to secondary rewards, consistent with previous studies showing reduced reward sensitivity in ASD youth (Damiano et al., 2015; Kohls et al., 2013). During negative feedback, youth with ASD traits showed reduced activation in the right medial PFC compared to those with low ASD traits. Previous studies in healthy adults showed that while obtaining an expected reward is associated with an increase in medial PFC activation, reward omission leads to decreased activation in this region (Knutson, Fong, et al., 2001; Knutson et al., 2003). This functional modulation was proposed to reflect medial PFC's role in tracking rewarding outcomes. In this context, our results may indicate that participants with high ASD traits find the lack of expected reward relatively more punishing or aversive (Tom, Fox, Trepel, & Poldrack, 2007) than those with low ASD traits. Combined with increased activation in reward-sensitive structures (putamen, thalamus, pallidum) (Haber & Knutson, 2010) in participants with high ASD traits following positive feedback, our results suggest that inadequate salience detection during reward anticipation may have led to exaggerated responses to both positive and negative reward outcomes.

Core ASD. As a plausibility check, we investigated the effects of anxiety and emotional problems on reward processing in participants with more strictly defined ASD symptoms, to ensure that our results were not simply accounted for by the threshold used to define the high ASD traits group. Despite considerably lower sample size, several of our results remained significant after defining ASD symptoms more strictly, including the following results in the comorbid group: hyperactivity in the right insula, thalamus and temporal regions during reward anticipation and reduced activation in lateral prefrontal regions following negative feedback.

Anxiety. Participants with anxiety showed increased activation in the right MFG during reward anticipation, but decreased right MFG activation following negative feedback, compared to participants without anxiety. MFG is part of the lateral PFC (Ridderinkhof et al., 2004), a region implicated in cognitive control via inhibition of prepotent behavioural responses (Burle, Vidal, Tandonnet, & Hasbroucq, 2004; E. K. Miller & Cohen, 2001; Stringaris, 2015). Increased activation in the right MFG during anticipation suggests that participants with higher anxiety symptoms required more cognitive effort to maintain stimulus-reward representations active when faced with competing events (E. K. Miller & Cohen, 2001). This is consistent with previous studies where anxious adolescents showed more emotional interference (Jazbec, McClure, Hardin, Pine, & Ernst, 2005) and heightened concern about making errors (Guyer et al., 2006) during reward processing compared to controls. However, we did not find the expected pattern of enhanced striatal activation during the anticipation of reward, which occurs specifically in social anxiety disorder (Forbes et al., 2006; Guyer, Choate, Pine, & Nelson, 2012).This could relate to the low rate of social anxiety in our sample. Conversely to reward anticipation, following negative reward feedback, anxious participants displayed reduced activation in the lateral PFC (right MFG and SFG, bilateral IFG) and bilateral caudate.

Interaction effects. The key aim of this study was to examine the interplay of ASD traits and anxiety symptoms during reward processing. We explored interaction effects to test whether the co-occurrence of ASD traits and anxiety was associated with a quantitative change in, or a qualitatively unique pattern of, reward-related brain activations. We found that youth with combined high ASD traits and anxiety showed a unique pattern of increased right insula activation during reward anticipation, as well as increased right MTG activation during anticipation and markedly low right-sided caudate, putamen, medial and lateral PFC activation during negative feedback. These effects remained significant after controlling for the effects of possible confounders (depression and ODD symptoms). Interestingly, insula hyperactivation was not observed in any of the main effects above, suggesting that youth with ASD traits and anxiety may display a qualitatively different pattern of neural activations during reward anticipation. The insula is implicated in “aversion-related” reward processing (Ernst & Fudge, 2009; Richards et al., 2013) particularly in anticipating and predicting the salience of aversive events (Chua, Krams, Toni, Passingham, & Dolan, 1999; Loewenstein, Weber, Hsee, & Welch, 2001; Ploghaus et al., 1999; Wittmann et al., 2014), as well as in interoceptive processing (Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004; Paulus & Stein, 2006; Seth, Suzuki, & Critchley, 2011). Interestingly, interoceptive prediction errors have been proposed to play a role in mood and anxiety disorders (Barrett & Simmons, 2015; Paulus & Stein, 2006) and theory of mind (Ondobaka, Kilner, & Friston, in press), while adults with ASD show significantly lower self-reported interoceptive awareness compared to controls (Fiene & Brownlow, 2015). Future studies should test directly whether the distinct pattern of activations observed during reward anticipation in our "combined"

group is related to interoceptive prediction errors, a possible etiological mechanism underlying the comorbidity between ASD and anxiety. Reduced right-sided caudate, putamen, and medial PFC (BA 10) activation during negative feedback suggests that participants in the combined group may have found not receiving the reward more aversive than other participants, similarly to reward anticipation. We also found reduced lateral PFC activation in the combined group following negative feedback. Interestingly, some but not all activation patterns that characterise the interaction effect were also found in the main effect of ASD traits (BA 10) and anxiety (right lateral PFC and right caudate), suggesting shared and unique neural substrates of negative reward feedback in youth with combined ASD traits and anxiety.

Longitudinal findings. In participants with high but not low ASD traits, increased right medFG and dorsal cingulate activations during reward anticipation were associated with increased likelihood of anxiety symptoms two years later. Predictions were significant after controlling for baseline anxiety showing that MRI can predict new onset of anxiety problems.

Irritability findings. In contrast to previous findings in children with SMD (Deveney et al., 2013), irritability symptoms in our study were not associated with changes in brain activation patterns following negative feedback. It is possible that our three-item measure was not sensitive enough to detect the true extent of trait irritability in the sample, leading to relatively low reported levels of irritability (e.g., mean irritability score for the high ASD traits group was 1.7 out of the maximum 6). However, the same measure was used successfully in a previous population-based study of irritability (Stringaris & Goodman, 2009b). Alternatively, the negative feedback condition in the present study may have been relatively less frustration-inducing than the one used by Deveney et al (2013), where participants lost some money accrued throughout the task when they made an incorrect response. In our negative feedback condition, participants failed to gain more points, but did not lose the points already won. The results suggest that at least in the context of our task, neural response patterns following negative feedback were associated with anxiety rather than with irritability.

Implications for comorbidity models. In Section 2.1.3 (page 51) I described different models that have been proposed to describe patterns of comorbidity between two or more co-occurring disorders. The present study is well-suited for investigating comorbidity models due to its 2x2 design, although the use of a single putative mechanism (reward processing) limits conclusions that can be drawn about the exact comorbidity model underlying the co-occurrence of ASD traits and anxiety (Banaschewski et al., 2007). Here, I will discuss how key findings from the present study may fit with various theoretical models of comorbidity outlined in Section 2.1.3, bearing in mind that our results will need to be replicated in an independent sample before making firm conclusions about the exact model underlying the co-occurrence of ASD traits and anxiety.

The *alternate forms* comorbidity model (Neale & Kendler, 1995) assumes that two comorbid disorders are alternative phenotypes with the same underlying pathophysiology. This model is not supported by our results, since we found differences in BOLD response to reward anticipation and feedback between the comorbid ASD_{ANX} group and "pure" ASD_{ONLY} and ANX_{ONLY}. This suggests that the three groups are not merely different manifestations of the same underlying pathophysiology.

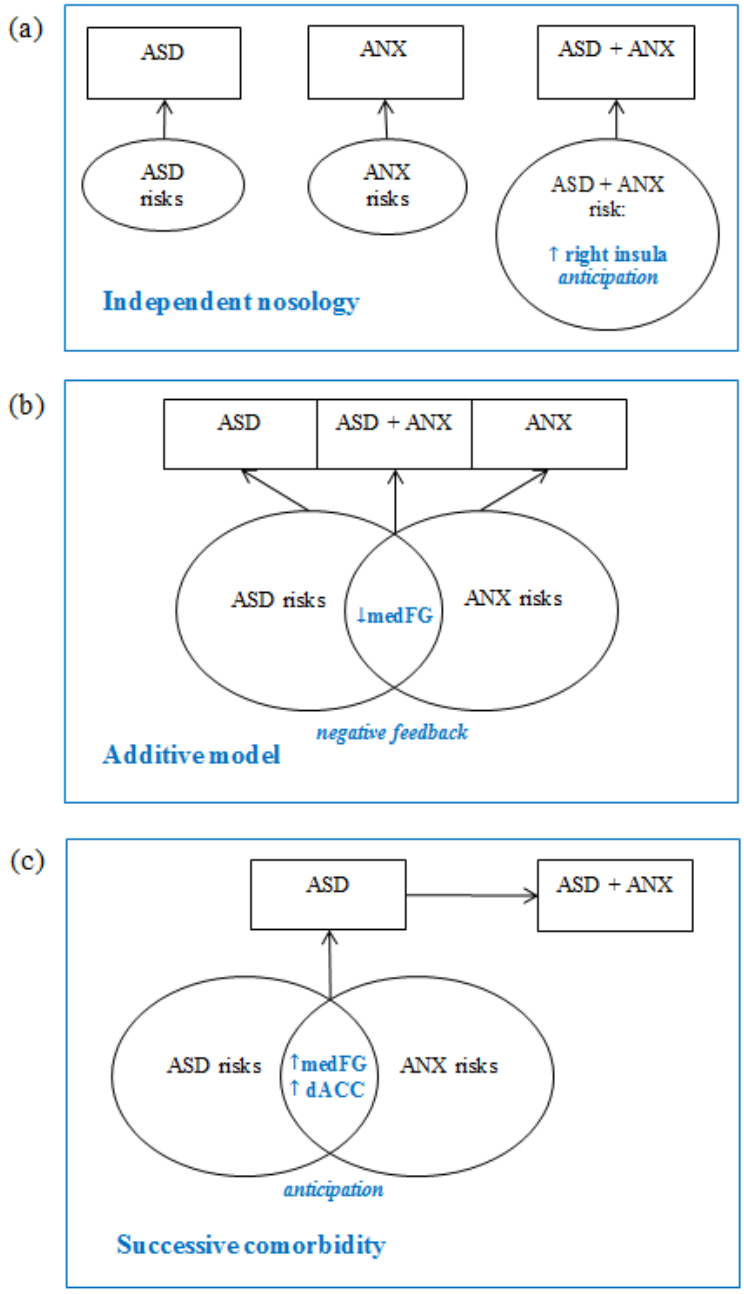
The *independent nosology* model (Banaschewski et al., 2007; Caron & Rutter, 1991) was also not fully supported, since we found common neural correlates for the main effects of ASD traits, anxiety and their interactions. For example, the right superior/medial frontal gyrus was less activated during negative feedback in those with high vs. low ASD traits, those with high vs. low anxiety symptoms, and the area was also present in the interaction analysis. While we did find a quantitative difference so that the right medFG was least activated in our comorbid ASD_{ANX} group, diminished activity in this area was a common mechanism across ASD_{ANX}, ASD_{ONLY}, and ANX_{ONLY}, arguing against the model of fully non-shared pathophysiology. One area that was found solely in the interaction effect was the right insula, which showed a distinctively high activation in the comorbid ASD_{ANX} group during reward anticipation (Figure 4.5 a). However, hyperactivation in the right insula did not emerge in subsequent t-tests (only left insula was found in $ASD_{ANX} > ASD_{ONLY}$ and $ASD_{ANX} > ANX_{ONLY}$ contrasts), limiting the robustness of the finding and suggesting the need of replication in an independent sample.

The fact that similar activation patterns were implicated in the main effects of ASD traits, anxiety, and their interaction suggests that comorbid ASD_{ANX} may represent a case where the independent effects of ASD traits and anxiety are somehow combined and/or amplified (Figure 4.5 b). This possibility should be investigated further using multiple constructs to compare the "pure" and comorbid ASD and anxiety (Banaschewski et al., 2007). In addition, it remains possible that some effects not found in the present study may emerge at higher levels of ASD and anxiety severity.

Finally, our longitudinal findings suggest that hyperactivation in right-sided medFG and dorsal cingulate during reward anticipation may represent a risk factor for developing anxiety in those with high ASD traits (Figure 4.5 c). In their paper outlining the pre-requisites for *successive comorbidity*, Caron and Rutter (1991) proposed that disorder "B" and comorbid A+B should share risk factors, and that the primary disorder "A" should be the one that generates these risk factors. This is consistent with our findings, whereby prefrontal activation patterns during reward processing predicted future anxiety in those with high ASD traits only ("disorder A"), unaffected by baseline anxiety ("disorder B"). To investigate the possible successive comorbidity in more detail, future studies should employ a longitudinal design where participants with ASD would complete a reward processing fMRI task at baseline and the underlying neural correlates would be compared between those who do vs. do not go on to develop anxiety later in time. We lacked

sufficient sample size to run such analyses, as only five participants with high ASD traits showed high anxiety symptoms at time 2 but not at baseline.

Figure 4.5. Schematic representation of how key results from our study may fit with theoretical comorbidity models presented in Chapter 2. See main text for a discussion.



ANX, anxiety. *ASD*, autism spectrum disorder. *dACC*, dorsal anterior cingulate cortex. *medFG*, medial frontal gyrus.

Clinical implications. Our findings suggest that the presence of combined ASD traits and anxiety is associated with both a quantitatively potentiated neural response to negative reward feedback (interaction showing a further reduction in prefrontal activations found in main effects) as well as emergence of qualitatively different neural correlates during reward anticipation (activation in right insula found exclusively in the interaction). This suggests that shared and distinct etiological

mechanisms might be involved in the comorbidity between ASD and anxiety, and, if replicated, carries important clinical implications. If the co-occurrence of anxiety in ASD is indeed underpinned by a distinct pathophysiological mechanism, the comorbid group may need to be recognised as a distinct nosological category and be researched in its own right. A specific biomarker of anxiety in ASD could aid differential diagnosis in cases where comorbid anxiety may be phenomenologically indistinguishable from ASD (Hartley & Sikora, 2009). Moreover, it is possible that medication response in the combined group is also different. We know already from ADHD literature that the effectiveness of methylphenidate is reduced in some youth with ASD (Simonoff, Taylor, et al., 2013) and in those with comorbid ADHD and anxiety (Moshe, Karni, & Tirosh, 2012; MTACooperativeGroup, 1999; Pliszka, 1989; E. Taylor et al., 1987). While existent evidence suggests that medications used in the treatment of anxiety disorders in the typically developing population also reduce anxiety symptoms in youth with ASD, e.g. SSRIs (Couturier & Nicolson, 2002; Kauffmann, Vance, Pumariega, & Miller, 2001; Namerow et al., 2003) or buspirone, which also decreased irritability symptoms in ASD youth (Buitelaar, van der Gaag, & van der Hoeven, 1998), the studies are limited by small sample sizes and often report case studies. An RCT investigating the effects of medication on anxiety in ASD vs. TD youth is still lacking.

Second, although MRI findings predicted only a small portion of the variance in new onset of anxiety at follow-up, brain activations were a significant predictor that could be used in establishing useful biomarkers of anxiety risk in youth with ASD traits. Our design strengthens the implication that the pattern of right-sided medFG and dorsal cingulate activations during reward anticipation is not merely a marker of anxiety, but may reflect an underlying mechanism by which young people with ASD traits become anxious. In addition, by measuring anxiety symptoms prospectively we avoid the limitation of recall bias, an important issue when studying successive comorbidity (Angold et al., 1999). Ultimately, finding an MRI biomarker of anxiety in ASD has a potential of guiding treatment interventions and measuring treatment response, especially useful in cases where the value of clinical interview is limited due to social communication difficulties.

Third, recent evidence suggests that disrupted processing of reward may lead to decision making problems (Sonuga-Barke, Cortese, Fairchild, & Stringaris, 2016; Viding & Seara-Cardoso, 2013). Future studies should investigate whether reward processing deficits can explain the presence of executive function deficits in ASD (Happé, Booth, Charlton, & Hughes, 2006; Shafritz, Bregman, Ikuta, & Szeszko, 2015), and explore the role of comorbid anxiety in the process.

Limitations. Although we investigated both anticipation and feedback phases of reward processing, task learning was performed outside of the scanner; therefore it was not possible to study the neural correlates of stimulus-reward learning. Second, due to sample size limitations we

were not able to fully distinguish between different types of anxiety disorders. Future studies should test whether the differential impact of specific types of anxiety on reward processing, seen in TD youth (Guyer, Choate, Detloff, et al., 2012; Kessel et al., 2015), holds in youth with ASD traits. In addition, the relative contribution of anxiety and ASD-specific difficulties on reward processing in youth with a clinical diagnosis of ASD, as opposed to sub-diagnostic ASD traits, remains to be studied. Finally, this study used parent report to assess symptoms of psychopathology in our adolescent sample. While used deliberately to keep the reporting source consistent with parent-rated ASD traits, some symptoms, especially those less overtly manifested, may have been missed by the parents, leading to underestimation of emotional problems in the sample.

In conclusion, over and above the independent effects of ASD traits and anxiety, we found qualitatively distinct and quantitatively potentiated neural correlates of reward processing in youth with combined anxiety and ASD traits. Future studies should assess whether the apparent co-occurrence of ASD and anxiety is associated with distinct etiological mechanisms.

Chapter 5 – Measurement of mood states in young people: Exploring the feasibility of ASL and pattern recognition

5.1 Abstract

Little is known about the neural correlates of mood states and the specific physiological changes associated with their valence and duration, especially in young people. Arterial spin labelling (ASL) imaging is particularly well-suited to study sustained cerebral states in young people, due to its robustness to low frequency drift, excellent inter-scan reliability and non-invasiveness. Yet it has so far been underutilised for understanding the neural mechanisms underlying mood states in youth. In this exploratory pilot study, 21 healthy adolescents aged 16 to 18 took part in an ASL mood induction experiment. Neutral, sad and happy mood states were induced using film clips and explicit instructions. Mood induction led to robust changes in self-reported mood ratings. Compared to neutral, sad mood was associated with increased regional cerebral blood flow (rCBF) in the left middle frontal gyrus and anterior prefrontal cortex, and decreased rCBF in the right middle frontal gyrus and the inferior parietal lobule. A decrease in self-reported mood from neutral to sad condition was associated with increased rCBF in the precuneus. Happy mood was associated with increased rCBF in medial frontal and cingulate gyri, the subgenual anterior cingulate cortex and ventral striatum, and decreased rCBF in the inferior parietal lobule. The level of current self-reported depressive symptoms was negatively associated with rCBF change in the cerebellum and lingual gyrus following both sad and happy mood inductions. Pattern recognition analyses revealed that sad and happy mood states can be reliably distinguished from neutral based on their associated rCBF patterns; however sad and happy moods were not accurately distinguished from each other. In conclusion, ASL is sensitive to experimentally induced mood changes in healthy young people. Future studies are needed to investigate the usefulness of ASL for detecting aberrant rCBF patterns associated with mood states in youth with psychopathology, including ASD and depression.

5.2 Introduction

So far in this thesis, I have focused mainly on anxiety and irritability, consistently identified as the most common co-occurring symptoms in young people with ASD. Importantly however, the detection of other comorbidities may be particularly limited by measurement difficulties, especially in cases where the condition is less likely to manifest outwardly, e.g. in depression (see Section 1.2.3 for a more detailed discussion). Indeed, depression prevalence estimates in ASD youth vary widely, and it has been suggested that reliance on parent-reported symptoms can lead to both under- and over-diagnosis of depression in ASD (M. E. Stewart et al., 2006; Walker & Stringaris, in preparation). In addition, there is a need for methods that would bypass the limitations of self-report in ASD, especially that around half of young people with ASD also suffer from global learning difficulties that weaken their ability to describe personal experiences and internal states (Elsabbagh et al., 2012; Grzadzinski et al., 2013).

One possible way of identifying depressed mood without relying on self- or parent-report is by investigating the brain activation patterns underlying mood states. However, so far we know little about the neural correlates of mood states and the specific physiological changes associated with their valence and duration, especially in young people. Here, we investigate these correlates using an fMRI technique known as arterial spin labelling (ASL) (Detre & Alsop, 1999) following the induction of neutral, sad, and happy moods in a group of healthy adolescents. We exploit the phenomenon of neurovascular coupling (Attwell et al., 2010) by measuring the changes in regional cerebral blood flow (rCBF) that accompany the onset and maintenance of specific mood states. We also employ pattern recognition techniques to investigate whether sad, happy, and neutral mood states can be distinguished from each other based on rCBF patterns alone, a particularly important step in the development of potential markers of mood states.

5.2.1 Advantages of using ASL to assess mood states

One reason for limited research into the neural substrates of mood is methodological. Experimental designs using fMRI typically involve stimulus change measured in the timescale of seconds (Matthews & Jezzard, 2004). It is also important to understand what underlies the persistence of mood states over sufficiently long periods given that the diagnosis and monitoring of patients requires measurement of psychopathology in the order of several hours to days and weeks. Time-series fMRI data using the blood-oxygen-level dependent (BOLD) contrast are sensitive to a low frequency drift, and thus less reliable when investigating neural activation changes over periods lasting longer than seconds (A. M. Smith et al., 1999). Electroencephalography (EEG), used to track brain activation changes over time, is not directly sensitive to sub-cortical neural activity (Kennett, 2012) and has poor spatial resolution, making it

less suited for investigation of limbic regions that are implicated in mood. Existing evidence on the neurophysiological correlates of mood states comes mainly from positron emission tomography (PET) studies that measured rCBF in participants experiencing experimentally-induced sadness or happiness (George, Ketter, Parekh, Herscovitch, & Post, 1996; Keightley et al., 2003; Liotti et al., 2002; Mayberg et al., 1999). However, the reliance of PET on radio-labelled compounds makes this method unsuitable when studying children and adolescents. This is unfortunate since mood disorders such as depression have their origins in adolescence, with a sharp increase in prevalence reported with the onset of puberty (Maughan, Collishaw, & Stringaris, 2013). Recent studies have also provided evidence for a link between adolescent depression and psychopathology later in life (Thapar, Collishaw, Pine, & Thapar, 2012). Therefore, discovering the physiological patterns associated with mood states in adolescence is particularly important.

ASL appears especially well-suited to studying the neural signatures of different mood states and their specific features, such as duration and intensity. First, since blood flow contrast is generated by the pair-wise subtraction of successively acquired pairs of images (see Methods section), the data is substantially free of low-frequency sources of contamination such as physiological noise and scanner drift, and less sensitive to subject movement (Aguirre, Detre, Zarahn, & Alsop, 2002; Detre & Wang, 2002; Howard et al., 2011; A. M. Smith et al., 1999). Second, the ASL pulse sequence acquisition parameters are tailored to maximize blood flow information from tissue capillaries and the data is therefore a more faithful signature of functionally-driven changes in neurovascular coupling (see Methods section). Unlike PET, ASL is non-invasive and has been previously used in children and newborns (Biagi et al., 2007; Wang et al., 2003). Furthermore, an ASL scan can be completed rapidly (<8 minutes), making it suitable for use with young people and clinical populations with limited ability to tolerate the scanning environment, such as children with ASD. ASL does not require a neuropsychological task which allows assessing those who are usually excluded from neuroimaging research, such as very young children or children with ASD whose performance may be compromised by task demands. ASL was already shown to successfully distinguish between states of depression in adults (Lui et al., 2009) and between adolescents with and without depression (Ho et al., 2013). In addition, its excellent inter-scan reliability (Hermes et al., 2007; Hodkinson et al., 2013) makes it suitable for monitoring treatment effects, which could be of particular importance in ASD youth where the reliability of symptom reporting using traditional methods is compromised.

5.2.2 Neural correlates of mood states: Evidence from studies of patients with depression and mood induction paradigms

One way of identifying neural correlates of mood states is to compare resting rCBF patterns between patients who are already depressed and healthy controls. Table 5.1 shows previous studies that used ASL for this purpose. Only one study to date has investigated rCBF in adolescents with depression, and none so far has studied rCBF patterns associated with depression in ASD youth. Ho and colleagues (2013) compared 25 medication-naïve adolescents with a current diagnosis of major depressive disorder (MDD; assessed with the K-SADS) and 26 healthy controls matched on a number of potential confounders, including age, gender, IQ, ethnicity, and socioeconomic status. They found that the groups differed in rCBF patterns in affective, executive, and motor networks. Increased sgACC perfusion in the depressed group (Ho et al., 2013) replicated a similar finding in adults with depression (Duhameau et al., 2010), consistent with PET evidence for increased sgACC rCBF in depressed patients that can be downregulated with antidepressant treatment (Drevets, Price, & Furey, 2008) and deep brain stimulation (Johansen-Berg et al., 2008). In addition, converging evidence points towards increased rCBF in the amygdala in adults with depression (Clark et al., 2006; Duhameau et al., 2010; Lui et al., 2009), although an opposite pattern was reported in adolescents (Ho et al., 2013). A major problem with translating findings from perfusion studies of patients to understanding the neural correlates underlying mood states is that the results may be confounded by considerable between-patients heterogeneity (e.g., disorder severity, duration, number of previous episodes, comorbidity, and medication use).

Mood induction is a more tractable method for an experimental study compared to studying patients who are depressed. Mood induction is an established experimental manipulation where the participant's mood state is temporarily changed. It can be used in within-subject designs, where each participant serves as his or her own control for different mood states, and specific characteristics of the stimulus, such as intensity, can be manipulated. Examples of methods used to elicit mood states include autobiographical recall, presentation of films, photos, music, or sentences (so-called Velten mood induction) of certain emotional valence, e.g. sad or happy. A meta-analysis concluded that showing film clips with explicit instructions to enter a specific mood state was the most effective method of inducing mood experimentally (Westermann, Spies, Stahl, & Hesse, 1996).

Mood induction has been used extensively in neuroimaging research (see Table 5.2); however most studies employed BOLD fMRI methodology that is suboptimal when investigating longer-lasting states, as discussed above. Despite its methodological advantages, ASL has only been used once with mood induction (Gillihan et al., 2010), in an adult sample. Most existing evidence for rCBF patterns associated with particular mood states comes from PET studies in TD adults. As shown in Table 5.2, some (Keightley et al., 2003; Liotti et al., 2002) but not all (George

et al., 1996; Gillihan et al., 2010; Lane, Reiman, Ahern, Schwartz, & Davidson, 1997; Ottowitz et al., 2004) studies in healthy adults found increased perfusion in the sgACC following sad mood induction. It is possible that autobiographical mood induction techniques employed by Keightley et al and Liotti et al were more effective in inducing sadness due to their self-referential nature, compared to sad faces and sentences, although this does not explain the lack of sgACC activation in Gillihan et al (2010) and Lane et al (1997) studies. Interestingly, Fontenelle et al (2012) reported that healthy adults showed increased connectivity between the sgACC and frontal regions, in particular the ventromedial PFC (vmPFC) and rostral ACC, during sad mood induction, consistent with these regions' involvement in the conscious experience of negative emotions in other fMRI (Berna et al., 2010; Goldin et al., 2005; Keedwell, Andrew, Williams, Brammer, & Phillips, 2005a; R. Smith et al., 2015) and PET studies (George et al., 1996; Mayberg et al., 1999). In addition, the amygdala is often activated in response to both sad (Furman, Hamilton, Joormann, & Gotlib, 2011; Gaffrey, Barch, Singer, Shenoy, & Luby, 2013; Goldin et al., 2005; Habel, Klein, Kellermann, Shah, & Schneider, 2005; Horacek et al., 2015; Joormann, Cooney, Henry, & Gotlib, 2012; Mitterschiffthaler et al., 2007; Schneider et al., 1995) and happy mood induction (Gaffrey et al., 2013; Gillihan et al., 2010; Habel et al., 2005).

To test the feasibility of examining mood states in youth using ASL, we investigated brain perfusion patterns involved in mood changes in a sample of healthy adolescents. We used film clips combined with mood elaboration instructions, a method found to be the most reliable in inducing mood (Westermann et al., 1996) and compared sad and happy mood conditions against the neutral. The inclusion of happy condition is based on its clinical relevance to depression, a disorder characterised not only by the predominance of sad mood, but also the absence or inability to perceive positive emotions (APA, 2013). fMRI studies show that adults with MDD display a dampened neural activation to positive stimuli relative to healthy controls (Epstein et al., 2006; Foland-Ross, Cooney, Joormann, Henry, & Gotlib, 2013). Also at the behavioural level, adults with depression achieve relatively lower ratings of happiness following happy mood induction compared to controls, and are less likely to maintain their positive affect even during the first minute of mood induction (Horner et al., 2014). It is therefore theoretically important to study the neural correlates of sad and happy moods together, as dysregulation of both these mood states is relevant to depression.

We hypothesised that the mood induction procedure will lead to significant changes in self-reported mood, and that it will generate rCBF changes in areas implicated in mood processing. We used unbiased, voxel-wise, whole brain analyses as well as predefined, bilateral regions of interest (ROIs): the amygdala, sgACC, dorsolateral prefrontal cortex (dlPFC), vmPFC, and the ventral striatum. We expected higher amygdala activation in response to emotional (sad or happy) than neutral conditions, based on its role in encoding emotional significance. We hypothesised that the sgACC would show higher activation following sad vs. neutral mood induction, based on previous PET mood induction studies in adults (Keightley et al., 2003; Liotti

et al., 2002; Mayberg et al., 1999) and sgACC hyperactivity in patients with depression (Drevets, Savitz, & Trimble, 2008), recently replicated using ASL in adults (Duhameau et al., 2010) and adolescents (Ho et al., 2013) with depression. Prefrontal ROIs were chosen based on their role in regulating limbic activity (Davidson, 2002; Drevets, Price, et al., 2008; Mayberg et al., 1999); therefore we expected these regions to be more activated in emotional (sad or happy) conditions compared to neutral. We also hypothesised increased rCBF in the ventral striatum following happy vs. neutral mood induction, based on a previous finding in healthy adults (Mitterschiffthaler et al., 2007) and the relation between ventral striatal activity and euphoria in healthy adults (Drevets et al., 2001). Finally, we explored whether the results are dependent on existing depressive symptoms.

5.2.3 Pattern recognition

While the first part of this chapter investigates rCBF patterns associated with different mood states using univariate methodology, in the second part we employ multivariate pattern recognition techniques to explore whether the three different mood states can be distinguished from one another based solely on rCBF patterns. Previously introduced in Section 1.2.3, pattern recognition techniques have been used successfully with fMRI to distinguish between children with ASD and TD controls (Jiao et al., 2010; Lim et al., 2013; Uddin et al., 2013), and between children with depression and healthy controls (Wu et al., 2015). However, no study has used the technique to investigate comorbid depression in ASD, possibly due to measurement difficulties discussed above. In this study, we take an important first step by testing the feasibility of pattern recognition combined with ASL for identifying mood states in TD youth. Research into the neural correlates of mood states has a potential to improve the diagnosis and monitoring of mood disorders in cases where diagnosis is uncertain, such as ASD.

Table 5.1. Studies that used ASL to compare resting rCBF between patients with depression and healthy controls.

Citation	Participants			rCBF: Main results in MDD (vs. controls)	Notes
	n	age group	medication use		
Colloby et al (2012)	68	elderly	yes	↑ white matter. No difference in grey matter rCBF (lateral and medial frontal, cingulate or parietal regions)	
Clark et al (2006)	25	adults	no	↑ right amygdala (ROI)	
Doraiswamy et al (1999)	19	elderly	yes	↓ left periventricular and parietal regions; rCBF in left frontal lobe approached significance.	Only 5 participants in the MDD group.
Duchameau et al (2010)	12	adults	yes	↑ sgACC, left dorsomedial PFC (BA 10), left dorsal ACC, left-sided putamen, pallidum and amygdala	Treatment-resistant MDD
Ho et al (2013)	51	adolescents (aged 13-17)	no	↓ parahippocampual gyri, inferior insula, IFG, right dlPFC, right ACC, cerebellum; ROI: right amygdala; ↑ right sgACC and right putamen, fusiform gyrus, ROI: superior insula.	
Järnum et al (2011)	43	adults	?	↓ frontal lobes and ACC in non-remitters vs controls	6-month follow up longitudinal study
Lui et al (2009)	79	adults	no	Non-refractory MDD: ↓ left MFG, ↑ right amygdala, right hippocampus, occipital regions. Refractory MDD: ↓ left MFG, right IFG, thalamus, left occipital regions/	
Orosz et al (2012)	44	adults	yes	↓ default mode network	
Ota et al (2014)	70	adults	yes	↓ right IFG, right ACC	All MDD patients were in remission
Roiser et al (2009)	21	adults	no	↓ anterior PFC (ROI) ↑ left-sided habenula and dlPFC (both ROI)	rCBF measured after acute tryptophan depletion
Shungu et al (2012)	28	adults	?	↓ left ACC, right lingual gyrus	
Vasic et al (2015)	72	adults	yes	↓ ACC, parahippocampal regions; ↑ frontoparietal and striatal regions	

(dl)PFC, (dorsolateral) prefrontal cortex. *IFG*, inferior frontal gyrus. *MDD*, major depressive disorder. *rCBF*, regional cerebral blood flow. *ROI*, region of interest analysis. *(sg)ACC*, (subgenual) anterior cingulate cortex. *?,* not reported. ↓, reduced. ↑, increased.

Table 5.2. Studies that combined mood induction procedures with neuroimaging.

Citation	Method		Participants				Main results (vs. neutral mood / baseline)
	MRI	Mood induction	n	groups	age group	medication use	
Berna et al (2010)	BOLD	Velten and music; sad vs. neutral	20	HC	adults	no	Sad: ↑ medial PFC (BA 10), OFC, rostral and perigenual ACC; ↓ left inferior temporal gyrus
Coen et al (2009)	BOLD	Music; sad vs. neutral	12	HC	adults	no	Sad: ↑ right insula, left SMA, thalamus, right MCC
Deckersbach et al (2008)	BOLD	Autobiographical text; sad vs. neutral	26	HC	adults	no	Sad: ↑ dlPFC, dorsal ACC, right IFG, right SMA, thalamus
Eugene et al (2003)	BOLD	Films; sad vs. neutral	20	HC	adults	?	Sad: ↑anterior temporal pole, right insula, OFC, medial PFC. NB. Large between-subject variability.
Farb et al (2010)	BOLD	Films; sad vs. neutral	16	MDD	adults	?	Sad: ↑ vmPFC, dmPFC, PCC, precuneus, caudate, left dlPFC, right hippocampus, right cerebellum; ↓ right insula, parietal regions, somatosensory cortex
Foland-Ross et al (2013)	BOLD	Sad film, then positive autobiographical recall	32	HC; remitted MDD	adults	yes	Both sad and happy: HC showed ↑ in left vlPFC and cuneus, but MDD showed ↓ relative to baseline (fixation cross).
Fontenelle et al (2012)	BOLD	Autobiographical recall; sad vs. neutral	21	HC; OCD	adults	yes	Sad: HC showed ↑ functional connectivity between sgACC and medial OFC and rostral ACC. OCD showed ↑ connectivity between sgACC and ventral striatum and hypothalamus.
Fortier et al (2010)	BOLD	Film clips; sad vs. neutral	90	HC (short vs. long 5-HTTLPR genotype)	children (mean age 8 years)	?	Sad: ↑ anterior temporal pole in both groups; left-sided insula, caudate and putamen in short allele group only.
Furman et al (2011)	BOLD	Film clips; sad vs. baseline (fixation cross)	49	HC (short vs. long 5-HTTLPR genotype)	adolescents (aged 10-15)	?	Sad: ↑ left amygdala (ROI) in short vs. long allele group.
Gaffrey et al (2013)	BOLD	Pictures of faces (40 sec per mood condition); sad vs. happy vs. neutral	54	HC; MDD	children (aged 4-6)	no	Both sad and happy: ↑ right amygdala and thalamus in MDD vs. HC (ROI)

George et al (1996)	PET	Autobiographical recall and faces; sad vs. happy vs. neutral	20	HC	adults	no	Sad: ↑ ACC, caudate, putamen, cerebellum, medial PFC, SFG, left insula. Happy: ↑ right caudate.
Gillihan et al (2010)	ASL	Script and autobiographical elaboration; sad vs. baseline (no task)	30	HC (short vs. long 5-HTTLPR genotype)	adults	?	Sad: ↑left ventral PFC in short allele group; ↑ vlPFC, ACC, insula in long allele group. ↑ precuneus in short vs. long allele group. Mood recovery: ↑ amygdala in short vs. long allele group.
Goldin et al (2005)	BOLD	Film clips; sad vs. happy vs. neutral	13	HC	adults	no	Sad: ↑ amygdala, left thalamus, IFG, medial PFC, precuneus, left lingual gyrus. Happy: ↑ medial PFC, right putamen
Habel et al (2005)	BOLD	Mood elaboration while viewing faces; sad vs. happy vs. gender discrimination baseline.	26	HC	adults	?	Sad: ↑ left-sided: amygdala, OFC, dlPFC, precuneus, insula, putamen. Happy: ↑ left-sided: amygdala, ACC, dlPFC.
Harrison et al (2008)	BOLD	Autobiographical recall; sad vs. neutral	24	HC	adults	no	Sad: ↑ functional connectivity between dorsal ACC and insula, ↓ functional connectivity of the DMN
Horacek et al (2015)	BOLD	Autobiographical script; sad vs. neutral, then viewed sad faces	40	HC vs. remitted bipolar disorder	adults	yes	Opposite amygdala responses in HC vs. bipolar groups. In HC, sad mood amplified amygdala responses to sad faces, while amygdala response was attenuated in patients.
Joormann et al (2012)	BOLD	Sad film, then positive autobiographical recall	47	HC vs. at-risk of MDD	children (aged 9-14)	no	Sad: ↑ amygdala and vlPFC in MDD vs. controls. Happy: ↑ dlPFC and dorsal ACC in MDD vs. controls.
Keedwell et al (2005a)	BOLD	Autobiographical prompt and faces; sad vs. happy.	12	HC	adults	no	Sad vs. Happy: ↑ vmPFC, ↓ right hippocampus
Keedwell et al (2005b)	BOLD	Autobiographical prompt and faces; sad vs. happy vs. neutral.	12	MDD	adults	yes	Sad: ↑ right-sided: insula, cerebellum. Happy: ↑ OFC, right vlPFC, right rostral ACC, cerebellum, left thalamus
Keightley et al (2003)	PET	Autobiographical text; sad vs. neutral	12	HC	adults	no	Sad: ↑ sgACC, PCC; ↓ dlPFC, dorsal ACC
Lane et al (1997)	PET	Film clips and autobiographical recall; sad vs. happy vs. neutral	12	HC	adults	no	Sad: ↑thalamus, caudate, putamen, cerebellum, temporal regions, hypothalamus, medial PFC (BA 9). Happy: ↑thalamus, BA 9, temporal regions.

Liotti et al (2002)	PET	Autobiographical script and mood elaboration; sad vs. baseline (eyes closed)	25	HC; MDD	adults	yes	Sad: In HC: ↑ sgACC, ↓ right BA 9 (PFC); In MDD: ↓ medial OFC/vlPFC (BA 10/11); In both HC and MDD: ↑ insula, cerebellum, ↓ PCC, IPL, inferior temporal cortex.
Malhi et al (2007)	BOLD	Word list sequences; sad vs. happy vs. neutral.	10	HC	adults	no	Sad: ↑ left insula, left caudate, superior frontal gyrus, PCC; Happy: ↑ left ACC and PCC.
Mitterschiffthaler et al (2007)	BOLD	Music; sad vs. happy vs. neutral	16	HC	adults	?	Sad: ↑right hippocampus/amygdala, left medial PFC, left cerebellum, left PCC; Happy: ↑ left-sided ventral striatum, caudate, medial PFC, ACC, PCC, precuneus
Ottowitz et al (2004)	SPECT	Velten; sad vs. neutral	8	HC	adults	no	Sad: ↑ left OFC, right ACC, right insula, cerebellum
Pagliaccio et al (2012)	BOLD	Film clip; sad vs. baseline (fixation cross)	55	HC vs. MDD history	children (aged 7-11)	no	Sad: ↓ left dlPFC, right superior frontal gyrus in those with MDD history vs. HC
Paulesu et al (2010)	BOLD	Faces (30 sec duration); sad vs. neutral	12	HC	adults	no	Sad: ↑ right dorsal ACC, right PCC, parahippocampal gyrus
Schneider et al (1995)	PET	Faces (40 per condition); sad vs. happy vs. fixation	16	HC	adults	no	Sad: ↑ left amygdala, ↓ left amygdala
Smith et al (2015)	BOLD	Music and pictures; sad vs. neutral	16	HC	adults	no	Sad: ↑ dorsomedial PFC, vlPFC, insula
Vrticka et al (2013)	BOLD	Film clips; funny vs. positive vs. neutral	22	HC	children (aged 6-13)	no	Funny: ↑ thalamus, PCC, IPL. Positive: ↑ IPL, right insula, temporal regions.

BOLD, blood-oxygen-level dependent. *(dl/vl/vm)PFC*, (dorsolateral/ventrolateral/ventromedial) prefrontal cortex. *HC*, healthy controls. *IPL*, inferior parietal lobule. *MCC*, midcingulate cortex. *MDD*, major depressive disorder. *OCD*, obsessive compulsive disorder. *OFC*, orbitofrontal cortex. *PCC*, posterior cingulate cortex. *PET*, positron emission tomography. *ROI*, region of interest analysis. *(sg)ACC*, (subgenual) anterior cingulate cortex. *SMA*, supplementary motor area. *SPECT*, single-photon emission computed tomography. *?,* not reported. ↓, reduced. ↑, increased.

5.3 Methods

5.3.1 Participants

22 healthy adolescents aged 16 to 18 (10 males, 12 females) were recruited via adverts on social media websites and internet fora for teenagers. In addition, one parent/carer of each participant completed a series of questionnaires (see below) about their child. One female participant was removed from subsequent analyses due to persistently high levels of anxiety whilst in the scanner, leaving a final sample of 21 participants. All participants were right-handed as measured by the Edinburgh Handedness Inventory (Oldfield, 1971). The adolescent participants did not have any serious medical, behavioural or emotional conditions, had no history of head injuries by self-report, and did not report any contraindication to MRI. Written informed consent was obtained from all participants. This study was approved by the Psychiatry, Nursing & Midwifery Research Ethics Subcommittee at King's College London (PNM/12/13-44).

Justification for sample size. The optimum number of subjects needed for a functional or perfusion MRI study is difficult to establish *a priori*, partly because the size of the desired effect is not known in advance (particularly in novel studies) and primarily because the MR signal in each of the voxels that cover the whole brain image has a large degree of spatial and temporal variability, depending on the differences in brain anatomy of each subject, spatial dependence of the sensitivity of the scanner coil, variability of neurovascular coupling over the whole brain, etc. Some studies suggest that 12 participants is sufficient to obtain good statistical power in most intra-individual design studies (Thirion et al., 2007). Studies estimating sample size required for ROI-based analyses using ASL suggest that the number of participants in this study is more than adequate (Murphy et al., 2011). The sample size also accounted for possible attrition.

5.3.2 Symptom assessment

Participants were screened for the presence of behavioural and emotional difficulties before taking part in the study with a series of self- and parent-reported questionnaires. Mood and Feelings Questionnaire (MFQ) (Costello & Angold, 1988) was used to measure depressive symptoms present in the previous 2 weeks. Symptoms of trait anger and irritability in the previous 6 months were measured using the ARI (Stringaris, Goodman, et al., 2012). Additional emotional and behavioural symptoms were measured using the Strengths and Difficulties Questionnaire (SDQ) (R. Goodman, 1997) that asks about symptoms in the last six months. Participants scoring high on any of these measures were excluded from the study. Individual cases were discussed

with a consultant child and adolescent psychiatrist (A.S.). Missing data were limited, with one parent-reported set of questionnaires not obtained.

5.3.3 Procedure

Stimuli. Based on a meta-analysis of mood induction procedures (Westermann et al., 1996), emotional film clips coupled with mood elaboration were chosen as a way of inducing mood. Each film clip was approximately 4 minutes long and depicted the following: neutral clip – a young man describing how to clip in and out of mountain bike pedals; sad clip – a scene from Dead Poets' Society (Weir, 1989) where a teenage boy finds out that his best friend committed suicide; happy – a series of stand-up comedy routines by a British comedian, Michael McIntyre. Before seeing each film clip, the participants were instructed to enter the specified mood state (as used previously by Habel et al., 2005). The instructions were as follows: "During this task, I would like you to try to become sad/happy. To help you do that, I will show you a video that most people find sad/happy". After seeing each film clip, the participants saw a message asking them to think about how the film had made them feel (as used previously, e.g. by Furman et al., 2011). For instance in case of sad mood condition, the instructions were as follows: "Have you ever been in a similar situation? Have you ever lost a loved one and if so, how did it make you feel? How would you feel if you were in the same situation?"

All participants were shown the scanner environment and invited to lay down inside our mock scanner in order to familiarise themselves with the scanning environment and reduce the potential for drop out. After confirming that they were ready to proceed, the participants entered the MRI scanner. First, a structural MRI scan was taken. The participants then rated their mood on a scale from 0 (very sad) to 10 (very happy), followed by the neutral mood induction that served as a baseline. After having watched the film clip, participants rated their mood again. This was followed by the first ASL scan (7:15min long, see below) during which the participants were instructed to remain still and look at the screen with the following text: "Think about how you felt when watching this neutral film clip. Please try to maintain this feeling while you're being scanned." The procedure was then repeated for the sad and happy conditions. Mood ratings for each condition were collected immediately after the end of each film clip.

MR imaging. The scanning was carried out at the Centre for Neuroimaging Sciences, Institute of Psychiatry, Psychology & Neuroscience, King's College London using a General Electric MR750 3.0T scanner.

In ASL, the MRI signal of endogenous arterial blood water is used as a contrast agent to measure rCBF. The contrast is achieved by 'labelling' or 'tagging' a bolus of arterial blood, by

inverting its magnetization in the region of the carotid arteries with an external (non-invasive) radiofrequency pulse. If two whole volume images are rapidly acquired in succession (one with and one without labelling of arterial blood), the resultant *difference* image is proportional to the volume of blood perfused into each unit volume of tissue during the time between the labelling and the acquisition of the image. This time is typically long enough (1.5s) so that the contrast is derived from labelled water in the micro-circulation (capillaries) and not in any of the larger arterioles. A suitable model is employed to convert the difference image into a map of rCBF in conventional physiological units of ml blood/100gm tissue/min. As stated earlier, the continuous pair-wise subtraction of labelled and non-labelled images makes ASL suitable for tasks using longer-lasting stimuli due to the low sensitivity to signal drift.

Each ASL image volume of 54 slices (3mm thickness, no interslice gap) was acquired using a pseudo-continuous flow-driven adiabatic inversion scheme (Dai, Garcia, de Bazelaire, & Alsop, 2008); TE/TR = 11.088/4901ms, flip angle (FA) = 111°, post-labelling delay 1525ms. Acquisition of five control and labelled pairs was done with a 3D FSE, multi-shot spiral stack, employing 8 spiral arms for each inter-leave in a total of 7:15min. Spiral k-space data was re-gridded to a 256 x 256 in-plane matrix prior to Fourier Transformation. A single proton density scan with the same acquisition parameters was used as a reference to compute rCBF in standard units. This procedure yielded rCBF maps with a resolution of 2x2x3mm. Enhanced fast gradient echo 3-dimensional sequence was used to collect T1-weighted images, with TR = 7.312 msec, TE = 3.016 msec, inversion time 400 msec, FA = 11^0, field of view = 270 mm, 256 x 256 matrix, 196 sagittal slices 1.2-mm thick.

5.3.4 Image processing

Image processing and analyses were performed using the Statistical Parametric Mapping suite (SPM, Functional Imaging Laboratory, University College London, London UK, version 8, www.fil.ion.ucl.ac.uk/spm). ASL images were normalised to the standard space of the Montreal Neurological Institute (MNI) by the following procedure: first, raw rCBF maps were co-registered to the high resolution T1-weighted anatomical volume after coarse alignment of the origin of both images. Segmentation of the T1-weighted image yielded a 'brain-only' binary mask which was multiplied by the co-registered rCBF map to produce an image free of extra-cerebral artefacts. Finally the T1-weighted image was transformed to the T1-weighted MNI template and the transformation parameters applied to the clean rCBF maps. All normalised rCBF maps were then spatially smoothed with a 8x8x8mm kernel.

ROI definition. ROIs (all bilateral) were defined using the WFUPickAtlas toolbox (Maldjian et al., 2003) available in SPM. The sgACC was defined as Brodmann area (BA) 25 and dilated by 1 voxel. The dlPFC was generated by combining BA 9 and BA 46, and was dilated

by 1 voxel. The amygdala was defined using the Automated Anatomical Labelling (AAL) library (Tzourio-Mazoyer et al., 2002). The vmPFC ROI combined bilateral medial orbital frontal and rectus regions from the AAL atlas. Since the atlas does not include a predefined mask for the ventral striatum, this ROI was defined as two 8 mm spheres based on MNI coordinates (right: x = 9, y = 9, z = –8; left: x = –9, y = 9, z = –8) derived from a previous meta-analysis (Postuma & Dagher, 2006) as used by Nusslock et al (2012).

5.3.5 Statistical analysis

Behavioural results. We first examined the effectiveness of our mood induction procedure in producing stimulus-congruent mood changes using repeated-measures ANOVAs and paired-samples t-tests on self-reported mood ratings.

Effects of mood induction on rCBF patterns. Whole-brain analysis of ASL images from the three mood induction conditions was performed using a one-way, within-subjects ANOVA with gender and mean global CBF added as covariates. This was due to small, but significant changes in global CBF that occurred during the time inside the scanner [$F(2,38)=8.66$, $p=.001$, $\eta_p^2=.313$; mean global CBF decrease from 56.2 to 53.9 ml blood/100gm tissue/min]. Since this was an exploratory study and to date there is no consensus regarding statistical analysis of ASL 'activation' data, we employed two different methods to indicate significance of findings at the whole brain level. First, we used the stringent, SPM-derived significance of $p<.05$ with family-wise error (FWE) correction based on cluster extent with a cluster forming threshold of $p < .01$ at the voxel level, as used in previous ASL studies (Pollak et al., 2015; Zelaya et al., 2011) and in the reward study in Chapter 4. Second, we performed Monte Carlo simulations using the AlphaSim program in Resting-State fMRI Data Analysis Toolkit (Song et al., 2011) to determine the cluster size (number of voxels) needed in order to achieve a corrected p lower than .05; thresholding the statistical images with a cluster-forming threshold of $p=.01$ and clustering with a cluster connection radius of 2mm. Minimum cluster size for all individual analyses are provided in the results section. For all ROI analyses, small volume correction in SPM was used, FWE-corrected at the voxel level.

Self-reported mood ratings and rCBF patterns. We then investigated whether the amount of self-reported mood change from neutral to sad/happy correlated with the amount of change in brain perfusion patterns. To do this, we performed a multiple regression analysis in SPM with the difference in self-reported mood scores (sad or happy minus neutral) regressed against the difference between respective perfusion images (neutral subtracted from sad or happy). Gender and mean global CBF were added to the model as covariates.

Effects of depressive symptoms, irritability, and anxiety. The effects of depressive symptoms on brain perfusion patterns following mood induction were examined using multiple

regressions in SPM. Total MFQ score was added to the model as a predictor, and the 'perfusion difference' image (neutral subtracted from sad or happy) as the outcome. Analogous regressions were performed for irritability using the ARI, and for anxiety using the SDQ emotional problems subscale. Gender and mean global CBF were added to the models as covariates.

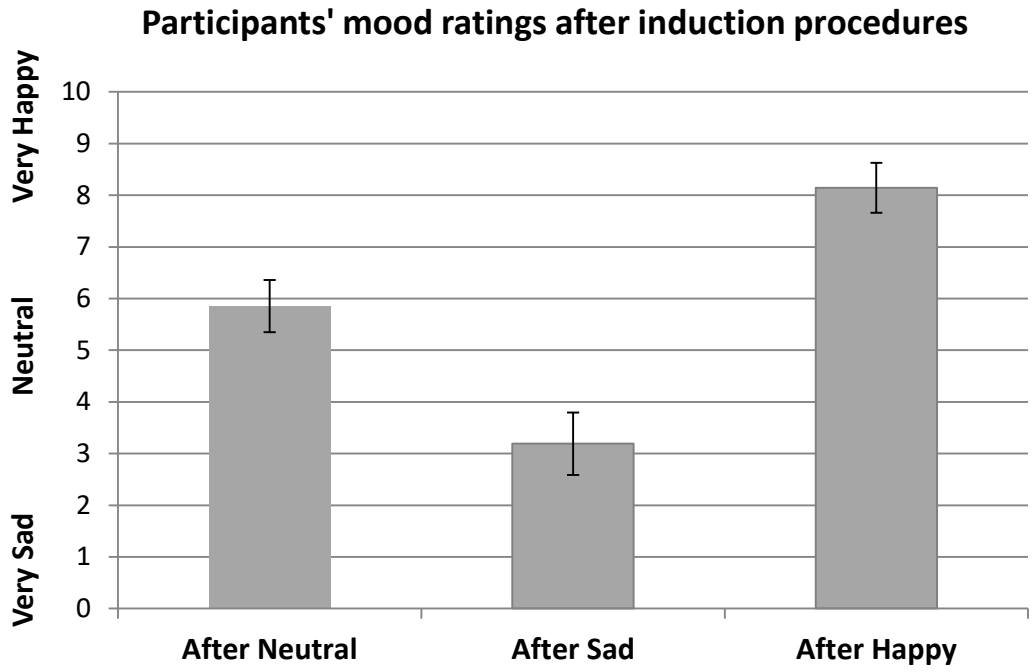
Multivariate pattern recognition. To perform multivariate comparisons between rCBF patterns underlying different mood conditions, we used a binary Gaussian process classifier (GPC) as implemented in the PIPR toolbox (King's College London, http://www.kcl.ac.uk/ioppn/depts/neuroimaging/research/imaginganalysis/Software/PIPR.aspx). GPCs are supervised multiple pattern recognition kernel classifiers, similar to the widely-used support vector machines (SVMs). GPCs provide predictive probabilities of class membership for unseen test data based on a set of 'training' data with labels of either +1 or -1 (for details, see Marquand et al., 2010). GPCs have been successfully used in previous studies (Doyle et al., 2013; Young et al., 2013), e.g. to distinguish between ADHD and ASD based on structural grey matter patterns (Lim et al., 2013). We used leave one-out cross validation (LOOCV) to assess the classifier's performance. LOOCV is an iterative process where data from all but one participant are used for 'training', and subsequently data from the remaining, withheld individual is used for testing. The reported classification accuracy is the mean accuracy value obtained across all LOOCV iterations, and it represents the degree to which two groups in each comparison can be distinguished based on their rCBF patterns. Whole-brain ASL perfusion images were used as input patterns and an a priori, SPM-defined grey matter mask was used. Statistical significance of classification accuracy was determined by permutation testing. To do this, the algorithm within PIPR software generated a distribution of permuted accuracies by re-training the classifier 1000 times, each time assigning class labels (+1 or -1) randomly to each image. The number of times that the permuted accuracy was higher than the true accuracy was then counted, and this number was divided by 1000 to provide a p value estimate for the classification. Classification was performed for the following possible configurations: neutral vs. sad, neutral vs. happy, and happy vs. sad. To visualise the pattern of rCBF driving the classification, multivariate discrimination maps (g-maps) were generated for the comparisons that were found to show significant classification accuracy (Marquand et al., 2010).

5.4 Results

5.4.1 Behavioural results

Mood ratings. As illustrated in Figure 5.1, the mood induction procedure led to significant changes in self-reported mood ratings among the participants, $F(1.41,28.10)=143.48$, $p<.001$, $\eta_p^2=.878$. Compared to the neutral condition, the participants rated their mood significantly lower after seeing the sad film clip, $t(20)=12.02$, $p<.001$, $d=2.18$, and significantly higher after seeing the happy clip, $t(20)=8.81$, $p<.001$, $d=2.10$. The difference in ratings between the sad and happy conditions was also significant, $t(20)=13.22$, $p<.001$, $d=4.19$.

Figure 5.1. Mean mood ratings (with 95% confidence intervals) from 21 participants after watching a neutral, sad and happy film clip in the scanner.



Questionnaire data. Total SDQ scores indicated average levels of emotional and behavioural difficulties in the sample by self- and parent-report (see Table 5.3). Anger and irritability levels were low by both self- and parent-report. All participants scored below clinical threshold for depression, and there was a strong cross-informant agreement between depressive symptoms as rated by the young people themselves and by their parents; r (20) = .50, p =.03.

Table 5.3. Means ± standard deviations (ranges) for mood and behavioural symptoms in the sample.

Depressive symptoms (MFQ)	
self-reported	6.9 ± 6.1 (0-22)
parent-reported	2.8 ± 2.8 (0-10)
Irritability (ARI)	
self-reported	1.6 ± 1.5 (0-6)
parent-reported	2.3 ± 2.5 (0-9)
Other symptoms (SDQ)	
Total SDQ	
self-reported	7.7 ± 3.9 (2-15)
parent-reported	4.9 ± 3.9 (0-12)
Emotional symptoms	
self-reported	2.3 ± 2.0 (0-6)
parent-reported	1.3 ± 1.2 (0-4)
Behavioural symptoms	
self-reported	1.3 ± 1.0 (0-4)
parent-reported	0.6 ± 0.8 (0-2)
Impact	
self-reported	0.1 ± 0.4 (0-1)
parent-reported	0.1 ± 0.3 (0-1)

5.4.2 Neuroimaging results

5.4.2.1 Sad mood

At whole brain level, sad mood induction led to increases in two clusters that were significant based on cluster-size AlphaSim threshold, but not FWE correction (Table 5.4 a, Figure 5.2 a), adjusted for the global CBF. The first, larger cluster with a peak in the left middle frontal gyrus also encompassed left postcentral gyrus. The second cluster included left medial superior frontal gyrus and BA 10. In contrast, right superior and middle frontal gyri and right IPL (BA 40) showed decreased rCBF after sad compared to neutral mood induction (Table 5.4 b, Figure 5.2 a). No significant ROI results were found.

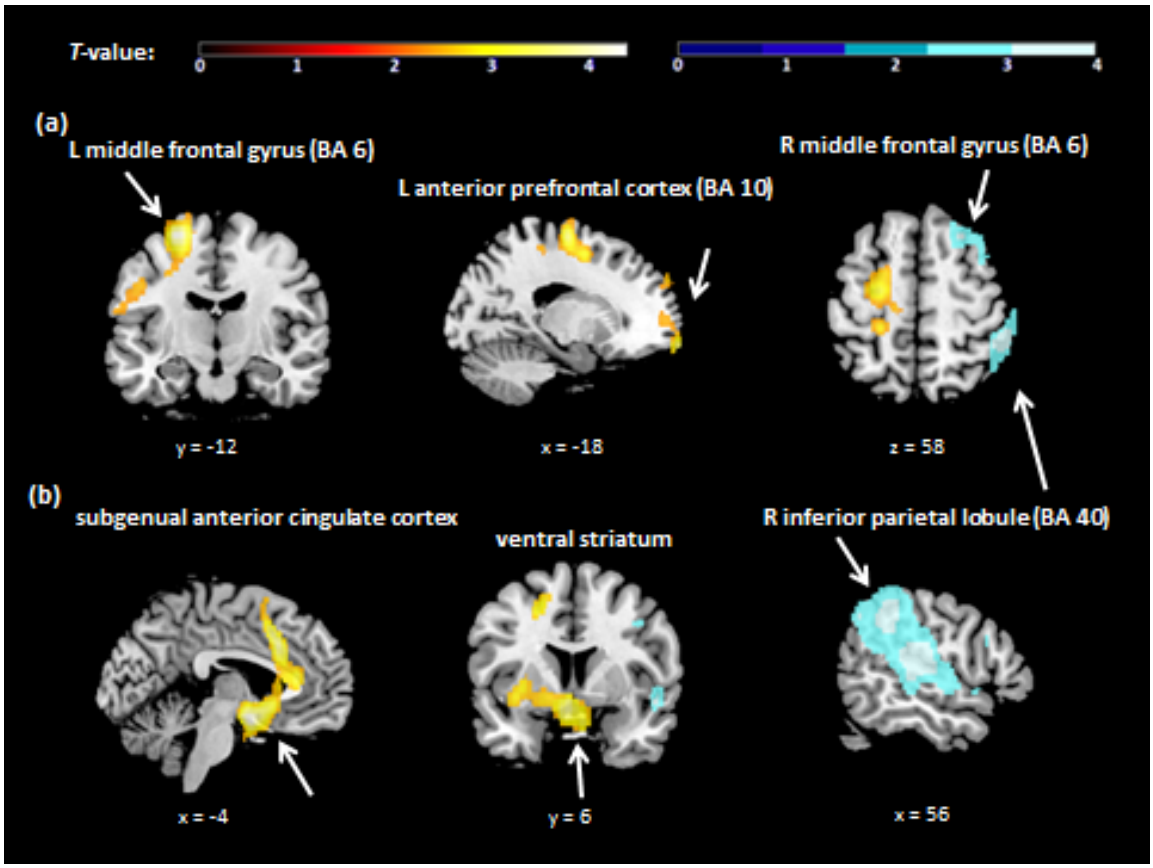
We then correlated the difference in brain perfusion patterns between sad and neutral mood induction conditions with the corresponding difference in self-reported mood ratings. We found a significant negative correlation in right precuneus at whole brain level (Table 5.4 c, Figure 5.3 a), suggesting that the decrease in self-reported mood from neutral to sad condition was associated with increased perfusion changes in this region. No significant ROI results were found.

Table 5.4. Whole brain level analysis results for (a,b) the ANOVA sad vs. neutral contrast, and (c) correlation between self-reported mood ratings difference and brain perfusion maps difference for sad minus neutral mood induction conditions.

Region	side	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)	AlphaSim corrected[1]
(a) Sad > Neutral								
middle frontal gyrus (BA 6),	L	1747	-24	-12	66	4.07	.231	$p_{corr} < 0.05$
postcentral gyrus			-20	-2	52	2.99		
			-64	-6	16	2.87		
medial superior frontal	L	744	-18	68	-14	3.40	.752	$p_{corr} < 0.05$
gyrus, anterior prefrontal			-14	58	4	3.03		
cortex (BA 10)			-16	62	36	2.81		
(b) Sad < Neutral								
superior frontal gyrus,	R	550	24	16	68	3.53	.875	$p_{corr} < 0.05$
middle frontal gyrus (BA 6)			40	8	62	2.61		
inferior parietal lobule (BA	R	1963	50	-42	58	3.45	.175	$p_{corr} < 0.05$
40)			44	-62	46	3.15		
			40	-68	40	3.11		
(c) Correlation with mood ratings								
Negative correlation								
precuneus	R	914	8	-80	52	3.54	.603	$p_{corr} < 0.05$
			14	-54	14	2.97		
			22	-84	48	2.87		

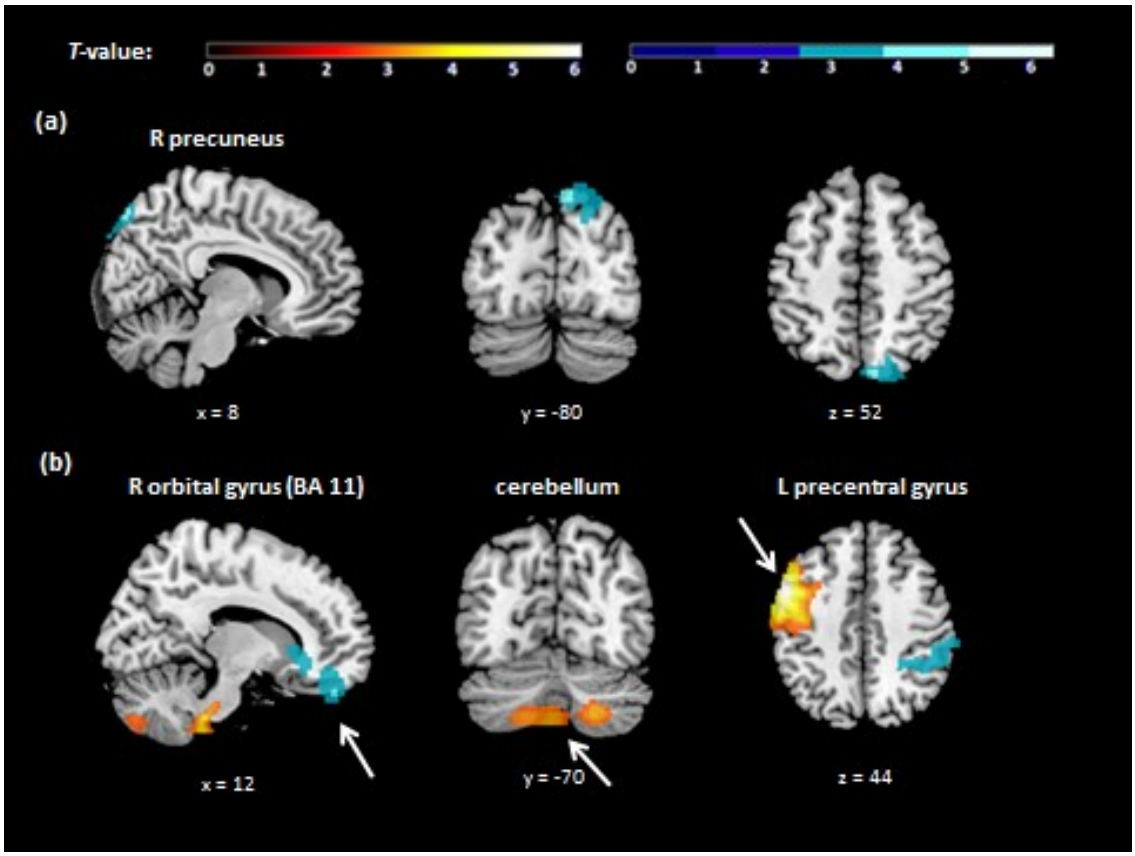
[1] Cluster extent threshold was 477 voxels for the ANOVA and 504 voxels for correlation analyses.
BA, Brodmann area. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere.

Figure 5.2. Whole brain level ANOVA results showing regional cerebral blood flow (rCBF) levels for the contrasts (a) sad vs. neutral and (b) happy vs. neutral, overlaid on a T1-weighted structural brain image. Orange = increased rCBF relative to neutral, blue = decreased rCBF relative to neutral. All locations are reported in MNI coordinates. For illustration purposes, the cluster-level significance is $p < .05$ (AlphaSim corrected).



BA, Brodmann area. *L*, left. *R*, right.

Figure 5.3. Results of whole brain level analyses for the regressions between self-reported mood rating differences and regional cerebral blood flow (rCBF) differences for the contrasts (a) sad minus neutral, (b) happy minus neutral, overlaid on a T1-weighted structural brain image. Orange = positive correlation, blue = negative correlation. All locations are reported in MNI coordinates. For illustration purposes, the cluster-level significance is $p < .05$ (AlphaSim corrected).



BA, Brodmann area. *L*, left. *R*, right.

5.4.2.2 The role of depressive symptoms

Next, we investigated whether the magnitude of neural activation following mood induction depended on the level of current depressive symptoms. We performed a regression analysis of 'perfusion difference' images (neutral condition image subtracted from sad or happy) against total MFQ scores.

For sad mood condition, we found negative whole brain level correlations between self-reported MFQ and the sad minus neutral perfusion difference. As shown in Table 5.5 a and Figure 5.4 a, higher MFQ scores were associated with lower rCBF in bilateral cerebellum, right lingual gyrus and right BA 18. The results were significant on the cluster-size, but not FWE-corrected, level. No significant ROI results were found.

We also found whole brain level correlations between self-reported MFQ and the happy minus neutral image difference. As can be seen in Table 5.5 b and in Figure 5.4 b, the higher the MFQ score, the higher the rCBF after watching the happy vs. neutral film clip in the SMA and a large cluster encompassing left middle and inferior temporal gyri. In contrast, MFQ scores were negatively correlated with rCBF in a large cluster encompassing the lingual gyrus and cerebellum (Table 5.5 b). No significant ROI results were found.

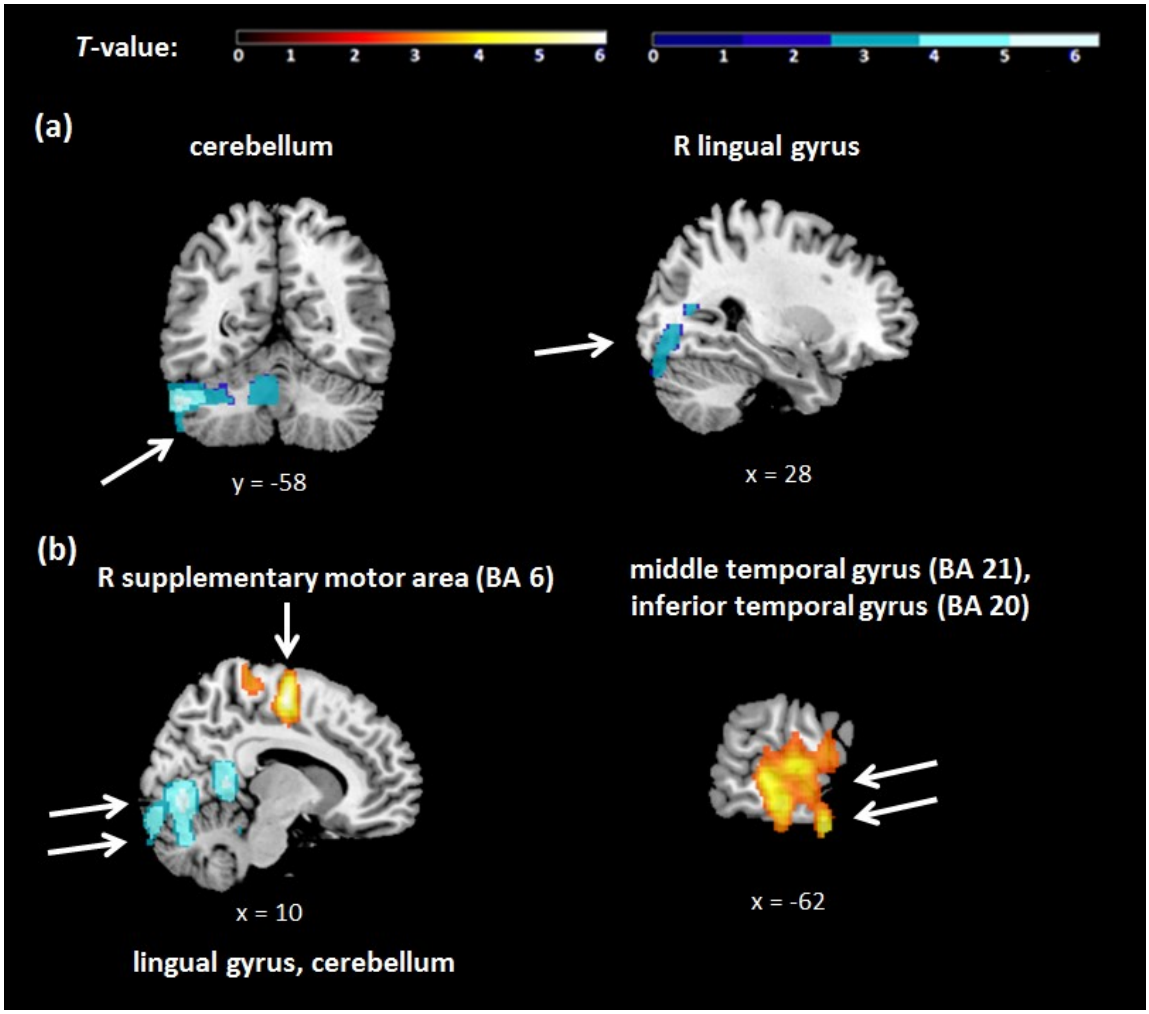
Table 5.5. Correlation results between self-reported depressive symptoms (MFQ) and brain perfusion maps difference for (a) sad minus neutral (b) happy minus neutral; all at whole brain level.

Region	side	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)	AlphaSim corrected[1]
(a) Sad mood induction								
Negative correlation								
cerebellum	L	1650	-54	-58	-34	4.63	0.218	$p_{corr} < 0.05$
			-24	-46	-38	3.50		
			-4	-52	-30	3.44		
cerebellum, lingual gyrus, BA 18	R	972	28	-88	-24	3.19	0.556	$p_{corr} < 0.05$
			24	-80	0	2.90		
			24	-68	12	2.89		
(b) Happy mood induction								
Positive correlation								
SMA (BA 6)	R	2443	10	-12	58	4.36	0.104	$p_{corr} < 0.05$
			4	-32	66	3.65		
			26	-22	66	3.25		
middle temporal gyrus (BA 21), inferior temporal gyrus (BA 20)	L	3497	-62	0	-28	3.78	0.030	$p_{corr} < 0.05$
			-62	-22	-16	3.62		
			-60	-30	-6	3.59		

Negative correlation							
Lingual gyrus, cerebellum	7503	22	-74	2	3.66	0.001	$p_{corr} < 0.05$
		32	-72	0	3.62		
		-14	-48	-36	3.46		

[1] Cluster extent threshold was 484 voxels for sad mood condition and 568 voxels for happy mood condition. *BA*, Brodmann area. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere. SMA, supplementary motor area.

Figure 5.4. Results of whole brain level analyses for the regressions between self-reported depressive symptoms (MFQ) and regional cerebral blood flow (rCBF) difference: (a) sad minus neutral, (b) happy minus neutral, overlaid on a T1-weighted structural brain image. Orange = positive correlation, blue = negative correlation. All locations are reported in MNI coordinates. For illustration purposes, the cluster-level significance is $p < .05$ (AlphaSim corrected).



BA, Brodmann area. *L*, left. *R*, right.

5.4.2.3 Happy mood

At the whole brain level, happy mood induction led to significant increases in rCBF in a large cluster extending from the brainstem via the cingulate gyrus to the medial frontal gyrus (see Table 5.6 a and Figure 5.2 b) and a smaller cluster encompassing the sub-gyral areas of left parietal and frontal lobes. By contrast, a large cluster including the inferior parietal lobule showed decreased rCBF after happy compared to neutral mood induction (Table 5.6 b and Figure 5.2 b). ROI analyses revealed increased rCBF in the sgACC and ventral striatum (see Table 5.6 a). There was also a marginally non-significant finding in the amygdala (p = .051).

Table 5.6. Whole brain and ROI results for (a,b) the ANOVA happy vs. neutral contrast, and (c) correlation between self-reported mood ratings difference and brain perfusion maps difference for happy minus neutral mood induction conditions.

Region	side	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	p (FWE)	AlphaSim corrected[1]
(a) Happy > Neutral								
Whole Brain Analysis								
brainstem, cingulate gyrus (incl. BA 32) , medial frontal gyrus		4461	-4 -6 -14	0 16 14	-16 42 50	3.89 3.56 3.53	0.009	$p_{corr} < 0.05$
Sub-gyral (parietal and frontal lobes)	L	477	-22 -28 -20	-36 -38 -40	54 38 40	3.52 3.47 2.75	0.913	$p_{corr} < 0.05$
ROI Analysis								
sgACC		507	-4	2	-16	3.81	0.005	
ventral striatum		271	-4	6	-12	3.38	0.010	
amygdala	L	13	-16	-8	-14	2.72	0.051	
(b) Happy < Neutral								
Whole Brain Analysis								
inferior parietal lobule (BA 40)	R	6112	56 54 48	-42 -24 -56	38 14 44	3.51 3.49 3.29	0.002	$p_{corr} < 0.05$
(c) Correlation with mood ratings								
Positive Correlation								
Whole Brain Analysis								

BA 8, precentral gyrus, MFG	L	5530	-54 -30 -48	6 -24 22	44 76 46	4.72 3.64 3.41	0.003	$p_{corr} < 0.05$
brainstem	R	940	18 14 0	-34 -34 -42	-50 -42 -50	3.55 3.54 3.11	0.626	$p_{corr} < 0.05$
cerebellum		1312	24 0 -18	-70 -74 -70	-44 -44 -46	3.06 2.95 2.77	0.410	$p_{corr} < 0.05$
superior temporal gyrus	L	561	-40 -42 -44	-44 -48 -42	16 8 28	2.83 2.81 2.79	0.870	$p_{corr} < 0.05$
ROI Analysis								
amygdala	L	78	-30	-2	-18	3.27	0.012	
dlPFC	L	1162	-54	6	42	4.71	0.002	
Negative Correlation								
Whole Brain Analysis								
orbital gyrus (BA 11), ACC, putamen	R	1241	12 8 24	48 30 18	-28 -6 4	3.17 3.01 2.98	0.446	$p_{corr} < 0.05$
inferior parietal lobule / postcentral gyrus (BA 40)	R	1653	46 52 52	-46 -34 -40	56 52 46	3.05 2.91 2.86	0.269	$p_{corr} < 0.05$

[1] Cluster extent threshold was 477 voxels for the ANOVA and 540 voxels for correlation analyses.
ACC, anterior cingulate cortex. *BA*, Brodmann area. *dlPFC*, dorsolateral prefrontal cortex. *FWE*, family-wise error correction. *L*, left hemisphere. *MFG*, middle frontal gyrus. *R*, right hemisphere. *ROI*, region-of-interest. *sgACC*, subgenual anterior cingulate cortex.

We then correlated the difference in brain perfusion patterns between happy and neutral mood induction conditions with the corresponding difference in self-reported mood ratings. We found a significant positive correlation in the BA 8, precentral gyrus, cerebellum and superior temporal gyrus at whole brain level, as well as the amygdala and dlPFC ROIs (Table 5.6 c and Figure 5.3 b), suggesting that the increase in self-reported mood from neutral to happy condition was associated with increased perfusion in these regions.

By contrast, there was a negative correlation between the magnitude of self-reported mood change and rCBF between happy and neutral conditions in right inferior parietal lobule and right BA 11.

5.4.2.4 The role of anxiety and irritability

For completeness, we investigated whether the magnitude of neural activation following mood induction depended on the level of anxiety and irritability in the preceding six months. We performed regression analyses of 'perfusion difference' images (neutral condition image

subtracted from sad or happy) against SDQ emotional subscale scores and ARI total scores, respectively.

Sad mood. We found a negative whole brain level correlation between self-reported anxiety and the sad minus neutral perfusion difference. As shown in Table 5.7 a, higher anxiety scores were associated with lower rCBF in the left middle occipital gyrus and precuneus following sad vs. neutral mood induction.

We also found negative whole brain correlations with self-reported irritability, whereby higher irritability scores were associated with lower rCBF in the right precuneus and left MTG after watching the sad vs. neutral film clip. The results were significant on the cluster-size, but not FWE-corrected, level. ROI analyses revealed a negative correlation between irritability and rCBF in the ventral striatum following sad vs. neutral mood induction; although this correlation was just below statistical significance ($p_{FWE\text{-}SVC}$=.053). There was also a significant whole-brain positive correlation between irritability and rCBF in the right hippocampus, as well as with the right amygdala in ROI analyses.

Happy mood. We found a negative correlation between irritability and rCBF in the right superior temporal gyrus (see Table 5.7 b). No effects of anxiety and no significant ROI results were found.

Table 5.7. Correlation results between self-reported anxiety or irritability and brain perfusion maps difference for (a) sad minus neutral (b) happy minus neutral.

Region	side	Cluster size (voxels)	peak MNI coordinates x	y	z	Z	*p* (FWE)	AlphaSim corrected[1]
(a) Sad mood induction								
Negative correlation								
Anxiety								
Middle occipital gyrus (BA 19), precuneus	L	902	-44 -30 -30	-84 -72 -92	22 36 26	3.24 3.01 2.99	.069	$p_{corr} < 0.05$
Irritability								
Middle temporal and lingual gyri	L	778	-46 -16 -20	-52 -58 -58	-2 -2 24	3.27 2.96 2.90	.090	$p_{corr} < 0.05$
precuneus	R	507	24 18	-40 -52	50 42	3.26 2.95	.164	$p_{corr} < 0.05$

ROI: ventral striatum		85	-6	6	-6	2.81	.053	
			-10	6	-2	2.80	.055	
Positive correlation								
Irritability								
hippocampus extending to the amygdala	R	656	30	-10	-16	3.33	.116	$p_{corr} < 0.05$
			26	-24	-32	3.29		
ROI: amygdala	R	87	30	-10	-16	3.33	.020	
(b) Happy mood induction								
Negative correlation								
Irritability								
Superior temporal gyrus (BA 41)	R	1703	52		-20	-2	3.15	.028	$p_{corr} < 0.05$
			56		-28	12	2.88	
			50		-34	-16	2.87	

[1] For sad mood condition, the cluster extent threshold was 499 voxels for analyses with anxiety and 483 voxels for irritability. For happy mood condition, the cluster extent threshold was 580 voxels for analyses with anxiety and 534 voxels for irritability. *BA*, Brodmann area. *FWE*, family-wise error correction. *L*, left hemisphere. *R*, right hemisphere.

5.4.2.5 Pattern recognition

Comparisons of the neutral condition with the two emotional conditions yielded high classification accuracies reflecting significant differences in rCBF patterns. The analyses revealed accuracies of 85.71% (p<.001) and 71.43% (p=.046) in distinguishing neutral images from happy and sad, respectively. However, the classifier was unable to significantly distinguish between happy and sad conditions, with a classification accuracy of 57.14% (p=.285).

Figure 5.5 illustrates the pattern of rCBF that drives the classification between the neutral and happy conditions (discrimination map). Intensity values illustrate the relative positive weight distributions (red - neutral) and negative weight distributions (blue - happy). As shown, the pattern of weights favouring the happy mood is represented in subcortical striatal regions (including the ventral striatum), orbitofrontal, and cingulate regions (including sgACC), whereas positive weight distributions representing the neutral mood are predominantly represented in the temporal, parietal, and posterior cingulate regions, as well as right-sided superior frontal regions.

Figure 5.6 shows a discrimination map that illustrates the pattern of weights driving the classification between the neutral and sad conditions. The pattern of weights favouring the sad mood (blue) is represented in the medial and orbitofrontal regions (including portions of the sgACC), left middle frontal gyrus, and limbic regions including the right hippocampus. Positive weight distributions (red) representing the neutral mood are predominantly represented in the middle and posterior cingulate regions, parietal, occipital regions and the cerebellum, as well as the right middle frontal gyrus. Multivariate t-maps were used to examine the significance of weight distributions and showed good correspondence with discrimination maps.

Figure 5.5. Multivariate discrimination map (g-map) showing regional distribution of positive weights (red) favouring the neutral condition and negative weights (blue) favouring the happy condition, from GPC analyses.

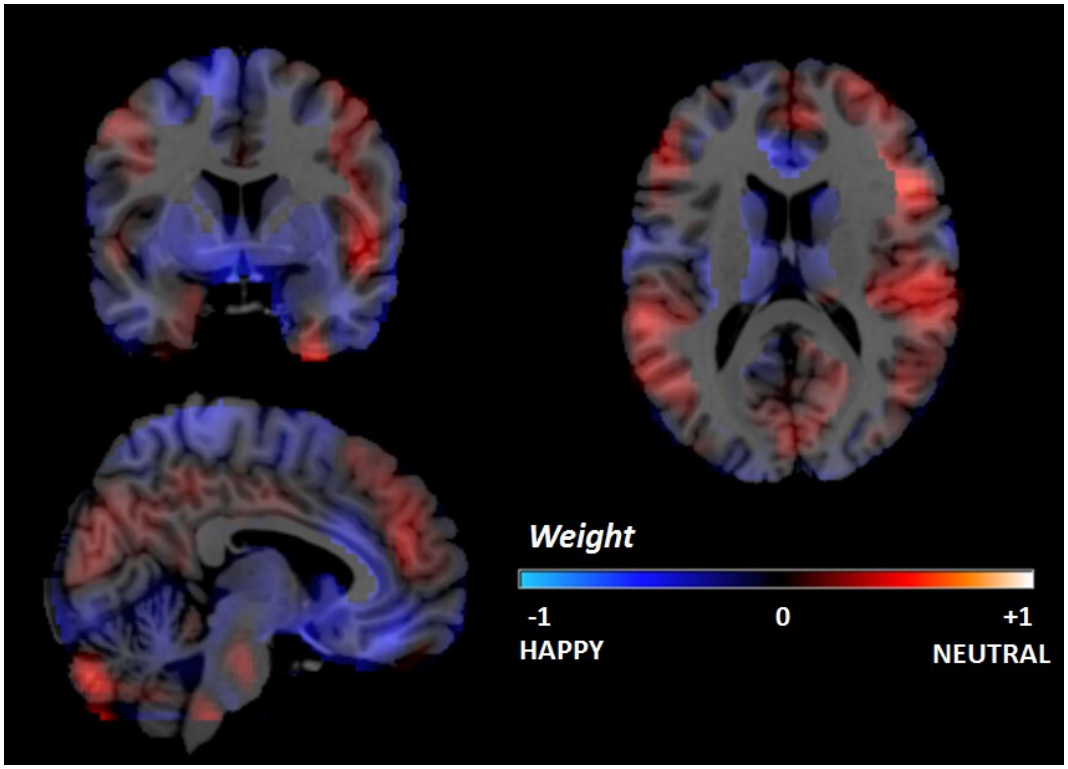
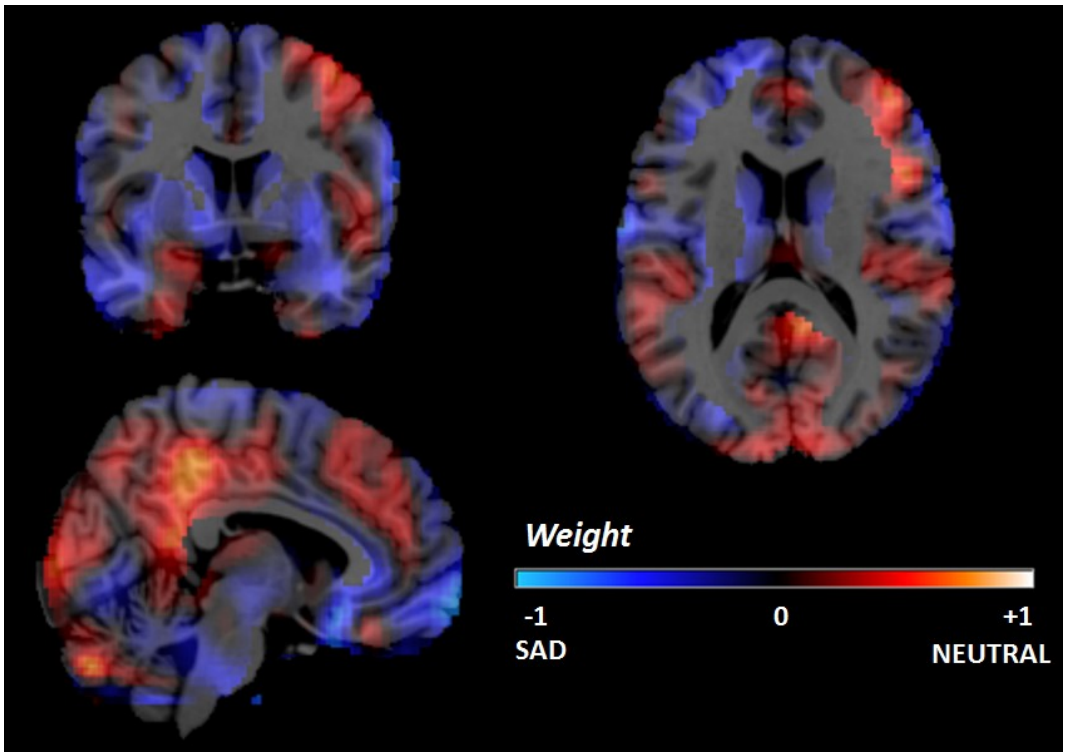


Figure 5.6. Multivariate discrimination map (g-map) showing regional distribution of positive weights (red) favouring the neutral condition and negative weights (blue) favouring the sad condition, from GPC analyses.



5.5 Discussion

This was the first exploratory study to investigate the neural substrates of mood states in young people using ASL, an MRI method that is especially suited to examining prolonged neural activation. We showed that mood changes can be robustly induced in healthy adolescents using our paradigm, as evidenced by significant changes in self-reported mood without significant between-subject variance. We also found rCBF differences following sad and happy mood induction procedures compared to neutral. The amount of rCBF change was affected by the degree of induced mood change and by depressive and irritability symptoms.

Sad mood. Our main finding in the sad vs. neutral contrast was a change in perfusion in the middle frontal gyrus (BA 6), with increased rCBF on the left, and decreased rCBF on the right side following sad mood induction. A PET study of adult depressed patients previously showed that decreased perfusion in middle frontal gyrus can be reversed with antidepressant treatment, consistent with the involvement of this region in mood processing (Buchsbaum et al., 1997). We did not expect lateralised findings, although one previous PET study in healthy adults also found rCBF in left middle frontal gyrus to be increased, and the right decreased, when performing a cognitive task following sad vs. neutral mood induction (Baker, Frith, & Dolan, 1997). Secondly, decreased rCBF in the inferior parietal lobule following sad vs. neutral mood induction is consistent with this region's role as a component of the default mode network (DMN), a network of brain regions that are active during wakeful rest (Buckner, Andrews-Hanna, & Schacter, 2008). Reduction in DMN activity has been associated with self-referential processing (Sheline et al., 2009). Crucially, we also observed decreased rCBF in the inferior parietal lobule following happy mood induction, suggesting that the DMN activity was suppressed when participants actively engaged in mood elaboration regardless of mood valence. Lastly, we found a correlation between the intensity of self-reported sadness and increased rCBF in the precuneus during sad mood elaboration, consistent with the role of precuneus in the recall of episodic and self-referential memory (Cabeza & Nyberg, 2000).

Sad vs. happy mood effects. Unlike in the happy condition, none of our sad mood induction findings reached the stringent, FWE-corrected significance level. There are two possible explanations. First, due to paucity of research with paediatric samples, our hypotheses were mainly based on PET mood induction studies with adults. These showed effects of both happy (George et al., 1996; Schneider et al., 1995) and sad mood induction on rCBF (Keightley et al., 2003; Schneider et al., 1995). It could be that adolescents show a weaker rCBF response to sad mood induction due to their stage of development or that there is higher variance in this response across subjects. A large-scale ASL study recently found that the trajectory of rCBF evolution

undergoes dynamic changes across adolescence (Satterthwaite et al., 2014). Consistent with the developmental hypothesis, Kliegel et al (2007) found that younger adults show lower emotional reactivity to negative mood induction compared to older adults, consistent with a weaker relation between daily stress and negative affect in younger vs. older adults (Mroczek & Almeida, 2004). However, the extent to which our results reflect a developmental effect is unclear without direct comparison using matched groups. Alternatively, the healthy, never-depressed adolescents included in this study might not be as susceptible to sad mood induction as they are to the happy. Using mood induction and fMRI, Joormann and colleagues found differences in neural activation between healthy girls and girls at risk of depression in areas implicated in negative mood processing (Joormann et al., 2012). We were unable to test this hypothesis directly since the MFQ scores of our participants were all within the non-depressed range. Future studies should investigate whether rCBF reactivity to sad mood induction is higher in adolescents with than without depression. Nevertheless, even in our non-depressed sample, we did find a negative correlation between the severity of depressive symptoms (self-reported MFQ score) and rCBF in the cerebellum and lingual gyrus following both sad and happy mood inductions. This is consistent with previous fMRI research showing decreased capacity for processing happy faces in the cerebellum and lingual gyrus in adults with depression (Fu et al., 2007), an effect that was reversed by antidepressant treatment. Decreased activity in the cerebellum and lingual gyrus in response to positive stimuli was also found in euthymic patients with bipolar depression (Malhi et al., 2007) compared to healthy controls. Together with the recent finding that adolescents with depression show decreased rCBF in the cerebellum compared to healthy controls (Ho et al., 2013), our results provide some additional evidence for the involvement of the cerebellum in emotional processing (Konarski, McIntyre, Grupp, & Kennedy, 2005). We also found a positive correlation between depressive symptoms and rCBF in the SMA following happy mood induction, although the role of this region in mood processing is unclear.

Happy mood. In line with our hypotheses, we found increased rCBF in the limbic regions (including the ventral striatum and a marginally not significant finding in the amygdala) following happy mood induction procedures. Moreover, there was a positive correlation between the self-reported increase in happiness and rCBF change in the left amygdala and left dlPFC. These results are consistent with the role of the fronto-limbic circuitry in emotional processing, with the amygdala involved in determining the emotional content of stimuli and frontal regions modulating emotional responses. This is in keeping with mood induction fMRI findings in patients with depression, who show an opposite direction of effects compared with healthy controls. For instance, in adults with MDD, severity of depressive symptoms correlated negatively with activation in the following areas following happy, but not sad mood induction: left putamen, bilateral caudate, left nucleus accumbens, and left amygdala (Keedwell et al., 2005b). Furthermore, decreased ventral striatum activity when processing positive words in depressed vs.

healthy adults correlated with symptoms of anhedonia (Epstein et al., 2006), consistent with abnormalities in the reward processing system in depression. Depressed adults also show an opposite pattern of vmPFC activation following happy and sad mood induction, compared to non-depressed adults (Keedwell et al., 2005a). Finally, adolescents with depression show lower rCBF in the dlPFC at rest compared to controls, as measured by ASL (Ho et al., 2013). Future studies should investigate whether adolescents with depression show dampened rCBF responsiveness to the happy – and heightened responsiveness to sad mood induction. Notably, happy mood induction is an ethically viable way of inducing a mood state, especially in children and those at risk of a mood disorder. Given what we know about decreased positive affect in depression, happy mood induction may function as a helpful probe for detecting depression in youth.

Contrary to our hypotheses, we also found an increase in sgACC perfusion following happy, rather than sad mood induction. sgACC activation to happy (as well as sad) stimuli was previously reported in adults with treatment-resistant depression (Kumari et al., 2003); where it was suggested that the severely-depressed patients responded to happy stimuli as to frustrative non-reward. However, our participants were not clinically depressed and we did not find a correlation between depressive symptoms and rCBF in the sgACC following happy mood induction in our sample. Interestingly however, healthy adults show significant functional coupling between the left amygdala and both the dlPFC and sgACC during emotion regulation (reappraisal of negative emotion) (Banks, Eddy, Angstadt, Nathan, & Phan, 2007). It is possible that co-activation of these regions in our study reflects the participants actively maintaining their happy mood following the induction procedure, although functional connectivity studies are needed to test this hypothesis (Fontenelle et al., 2012).

Effects of irritability and anxiety. While trait anxiety did not affect rCBF patterns substantially, we found that higher irritability scores were associated with higher rCBF in the right hippocampus (whole-brain analysis) and right amygdala (ROI) following sad vs. neutral mood induction. This suggests that those high on trait irritability were more likely to show limbic activation when consciously processing negative affect. The importance of irritability in affective processing is further suggested by the negative correlation between irritability and rCBF in the ventral striatum following happy mood induction (ROI, p=.053). The association between irritability and sad mood processing is particularly notable in light of the widely-reported cross-sectional and longitudinal relationships between irritability and depression, whereby early irritability predicts depression later in life (Rowe et al., 2010; Stringaris et al., 2009; Stringaris & Goodman, 2009a, 2009b). Although no firm conclusions can be drawn from the present study alone, future studies should investigate whether activity in the hippocampus during sad mood processing may be the mechanism underlying emergence of later depression in highly-irritable youth. One hypothesis is that the hippocampus, due to its involvement in encoding episodic memories and self-referential information (Lou et al., 2004; Northoff et al., 2006), could be involved in maladaptive rumination

that is a known predictor of depression (Nolen-Hoeksema, 2000; M. S. Robinson & Alloy; Stone, Hankin, Gibb, & Abela, 2011). Indeed, activation the hippocampus (NB. left-sided) was positively correlated with the intensity of self-reported angry rumination following experimental provocation in healthy undergraduates (Denson, Pedersen, Ronquillo, & Nandy, 2008).

Pattern recognition. Finally, we explored whether sad, happy, and neutral mood states can be distinguished from one another based solely on rCBF patterns. We found that neurophysiological responses to different mood induction conditions differentiated between neutral vs. sad and happy states. rCBF patterns driving the classification of happy mood were predominantly represented in the orbitofrontal, cingulate, and striatal regions, whereas neutral mood in the temporal, parietal, and posterior cingulate regions. Corresponding rCBF patterns for sad mood were represented in the medial, orbitofrontal, and limbic regions. However, the multivariate classifier failed to distinguish between rCBF patterns underlying sad vs. happy mood states. This could be due to the overlap of underlying rCBF patterns between the two conditions, particularly in the prefrontal regions. Indeed, previous fMRI studies in healthy adults also reported several prefrontal areas that were activated during both sad and happy mood inductions, including the vlPFC (Foland-Ross et al., 2013), medial PFC (Goldin et al., 2005; Lane et al., 1997; Mitterschiffthaler et al., 2007), and dlPFC (Habel et al., 2005). Our study extends the existing evidence by showing that multivariate pattern recognition techniques may not be useful in all scenarios, particularly when more subtle or isolated differences may be at play. Due to the exploratory nature of this study, and the lack of existing evidence for specific rCBF patterns associated with mood states in youth, our pattern recognition analysis was whole-brain-based. Future studies may benefit from identifying more localised circuits that are directly involved in rCBF changes underlying mood states in youth, and use these circuits as a pre-specified ROI mask for multivariate pattern recognition analyses. Alternatively, connectivity analysis methods may be useful in establishing whether functional coupling between regions, rather than different neural networks, underlie the differences between mood states.

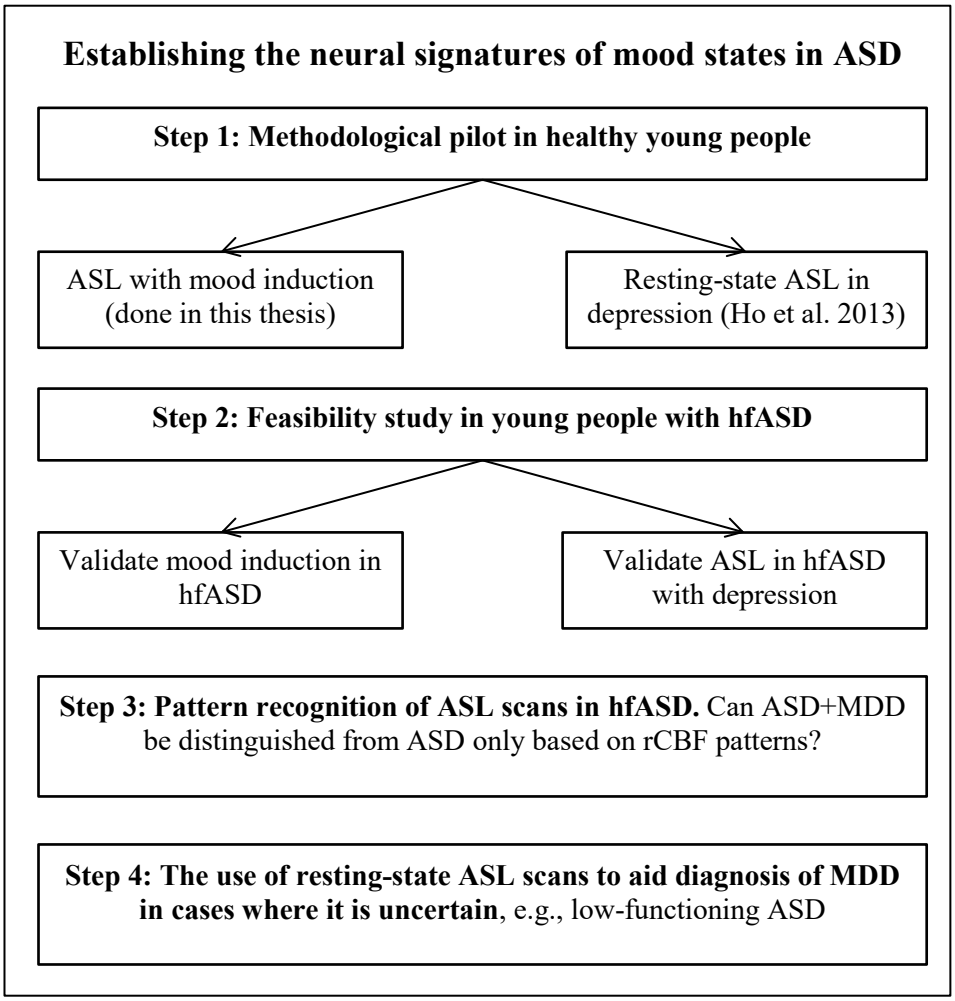
Implications for research in ASD youth. As noted previously, this study explored the methodological feasibility of combining ASL with mood induction to examine the rCBF patterns associated with different mood states; hence we used a healthy control sample. Our findings add to the existing evidence from one resting-state ASL study conducted in young people with depression (Ho et al., 2013), and represent a first step in the process of identifying the neural signatures of mood states in the ASD population (see Figure 5.7 on page 187). To this end, I mentioned in the introduction to this chapter why ASL may be especially well-suited for investigating rCBF patterns in the ASD population, including the rapidness of acquiring an ASL scan and the fact that ASL does not require a neuropsychological task. However, several challenges pertain to the research on rCBF patterns associated with mood states in young people

with ASD. The main challenge concerns the feasibility of mood induction in children with ASD, which has not been investigated so far (Step 2 in Figure 5.7). It is possible that the need to introspect on one's own mood state, required in the present experiment, would be problematic for youth with ASD, especially those with alexithymia. An alternative approach would be to investigate resting-state rCBF patterns in young people with ASD and co-occurring depression. However, disadvantages of this methodology were mentioned in Section 0, and one would need to additionally select young people with ASD where the diagnosis of depression is unlikely to reflect a measurement error. Should these challenges be overcome, e.g. by conducting the research in the high-functioning subsample of ASD youth, the third step in the process of identifying the neural signatures of mood states in ASD would involve pattern recognition (see Figure 5.7). This line of research would build on previous fMRI evidence where pattern recognition successfully distinguished between ASD and TD youth (Jiao et al., 2010; Lim et al., 2013; Uddin et al., 2013), and between children with vs. without depression (Wu et al., 2015). The crucial comparison would be to test whether young people with combined ASD and depression can be distinguished from ASD youth without depression based solely on rCBF patterns. Should this be possible, resting-state ASL scans may prove useful in aiding the diagnosis of depression in young people with ASD more generally (Step 4 in Figure 5.7). Overall, several challenges remain to be overcome before the neural signatures of mood states in young people with ASD can be firmly established.

Strengths and limitations. The main strength of this study is the combined use of ASL and mood induction to directly examine rCBF patterns associated with three different mood states in adolescents. Importantly, we carried out the scanning after film clip presentation, ensuring that the resulting neural activity reflected the participant's mood state and not film clip characteristics (colour, brightness, or sound). This study is limited by the fixed order of mood induction conditions, used deliberately to maximise the power to detect mood-specific rCBF patterns in our sample of 21 participants. A larger study with a randomised order of mood induction conditions is needed to rule out the possibility of an order effect or emotional contagion. Second, we did not collect resting-state fMRI data that would allow the investigation of functional connectivity between specific brain regions. Third, our multivariate pattern recognition method did not allow for the investigation of gender effects or trait markers on the classifier's performance, both of which could act as confounders in our machine learning analyses. Finally, with regard to in-scanner self-reported measures that tested the effectiveness of our mood induction procedure, we only used measures of sadness/happiness. Anger is an additional mood state that could also have been elicited by the procedure, especially in light of our findings with trait irritability. In addition, a general limitation of mood induction methods is that self-reported mood ratings may be influenced by the participants' desire to please the examiner.

In conclusion, our results show that ASL is sensitive to mood state in terms of absolute changes in rCBF, but more subtle changes across the brain characterise the sad mood state as compared to happy, evidenced by the pattern recognition analysis. The current study offers a crucial starting point for the investigation of mood states, using methodology that bypasses the limitations of conventional fMRI. Although important challenges remain (Savitz, Rauch, & Drevets, 2013), studying the tonic activation of neural networks involved in mood processing is likely to have important clinical implications for disorders characterised by persistently sad or happy mood, such as unipolar and bipolar depression, across development.

Figure 5.7. Future directions for research on the neural correlates of mood states in young people with ASD. See main text for a discussion.



ASL, arterial spin labelling. *(hf)ASD*, (high-functioning) autism spectrum disorder. *MDD*, major depressive disorder. *rCBF*, regional cerebral blood flow.

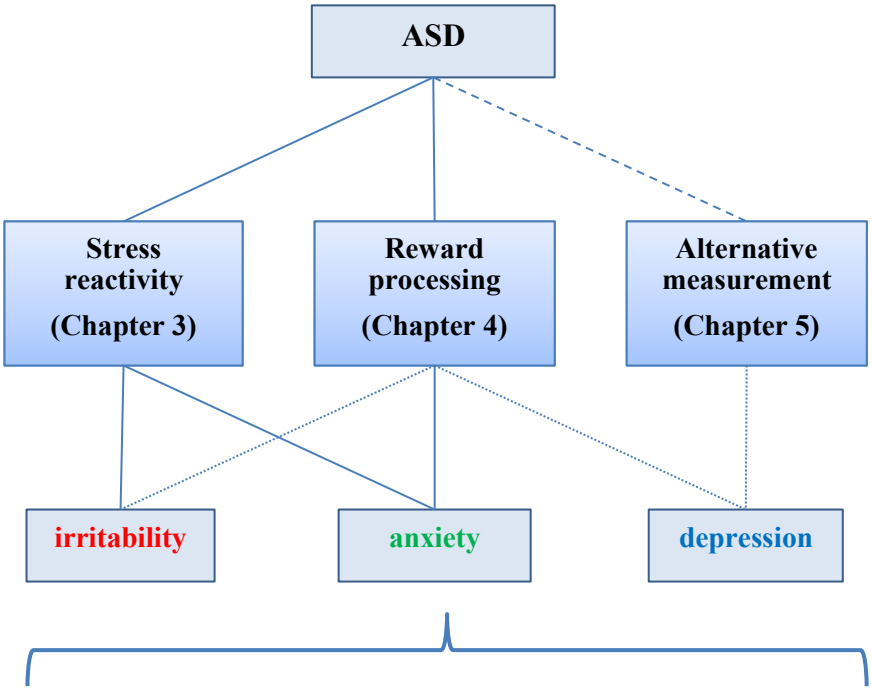
Chapter 6 – General discussion and conclusions

This thesis aimed to address the gaps in existing literature that pertain to the measurement and neurophysiological mechanisms of mood and anxiety problems in young people with ASD. In this final chapter I relate our findings back to the aims and objectives set out in the introduction. Following a summary of the main results of each individual study, I discuss how alterations in reward processing and physiological reactivity may underlie some of the mood and anxiety problems observed in youth with ASD. Study limitations and implications for comorbidity models, symptom measurement, and clinical practice are discussed, with directions for future research presented throughout the following sections.

6.1 Summary of results

An overview of key findings from this thesis is presented in Figure 6.1 on the following page. Below, I discuss the results from individual thesis chapters in more detail.

Figure 6.1. Summary of results from the three studies in this thesis. Different colours indicate findings relevant to particular symptoms comorbid with ASD: irritability, anxiety, or depression.



Stress reactivity

- Equal rates of subjective stress response in ASD and TD participants
- Irritability associated with reduced cortisol stress reactivity in ASD, even after controlling for anxiety
- Irritability associated with reduced HR stress reactivity and overall higher parasympathetic modulation in ASD but lost significance after covarying for anxiety
- Anxiety related to overall lower sympathetic activity during stress test, no effect of irritability.

Reward processing

- ASD traits associated with lower prefrontal activations during reward processing
- Main effect of anxiety: right MFG activation increased at anticipation but reduced at negative feedback
- ASDanx associated with insula hyperactivation during anticipation and frontostriatal hypoactivation following negative feedback
- Right-sided medFG and dACC activations predict new-onset anxiety in those with high ASD traits only
- Most findings not accounted for by ODD or depression.

Alternative measurement

- Mood induction led to robust self-reported mood changes
- ASL was sensitive to detecting rCBF patterns associated with different moods
- The effects of happy mood induction on rCBF patterns were generally stronger than the effects of sad mood
- Pattern recognition analyses distinguished sad and happy moods from neutral, but not from each other, based on their rCBF patterns.

ASD, autism spectrum disorder. *ASDanx*, combined ASD traits and anxiety. *ASL*, arterial spin labelling. *dACC*, dorsal anterior cingulate cortex. *HR,* heart rate. *medFG*, medial frontal gyrus. *MFG*, middle frontal gyrus. *ODD*, oppositional defiant disorder. *rCBF*, regional cerebral blood flow. *TD*, typically developing.

6.1.1 Chapter 3 – Stress reactivity

Chapter 3 focused on physiological reactivity to psychosocial stress in young people with ASD. We tested the hypothesis that stress responsiveness in ASD is associated with irritability. We concurrently assessed the effects of anxiety since anxiety and irritability tend to co-occur in ASD (Simonoff et al., 2008; Tantam, 2003) and because anxiety may itself be associated with distinct physiological patterns of stress response (see Section 2.3.3.2).

We first investigated whether irritability can be measured reliably in ASD using the ARI, an irritability scale developed in TD youth. Self- and parent-reports of irritability were compared between 47 boys with hfASD, 40 boys with SMD, and 30 HC boys. We found a similar pattern of irritability symptom reporting across the hfASD and SMD groups, by both parent- and self-report. Parent- and self-reports of irritability in the hfASD sample were strongly correlated with each other and both showed high internal consistencies. The reported impairment due to irritability was directly proportional to irritability symptom severity across informants, in keeping with results in TD youth. Overall, the results suggest that boys with hfASD are able to report reliably on these problems; moreover, the hfASD boys in this sample experienced high and impairing levels of irritability.

We then examined physiological stress responses in 47 boys with hfASD and 23 TD boys, and the relative influence of irritability and anxiety on physiological stress reactivity in boys with hfASD. Boys with hfASD showed a statistically significant increase in self-reported stress following the PST which was indistinguishable from that of TD boys. Cortisol levels, HR, and HRV changed significantly during the stress test in both groups, but cortisol and HR reactivity were steeper in TD compared to hfASD boys.

Cortisol reactivity in hfASD. Boys who were rated as highly-irritable by their parents showed a blunted cortisol stress response, while boys who rated themselves as highly irritable displayed lower levels of cortisol both pre- and post-stressor. The findings remained significant after controlling for anxiety and no independent effects of anxiety on cortisol response were found when anxiety and irritability were assessed together. Therefore, cortisol stress-responsiveness in boys with hfASD seemed specifically related to irritability.

HR and HRV reactivity in hfASD. Similarly to cortisol findings, boys rated as highly-irritable by their parents showed a blunted HR reactivity to psychosocial stress. However, this finding was no longer statistically significant after controlling for anxiety; instead, high parent-reported anxiety was associated with a dampened HR response to stress. A main effect of parent-reported irritability on HRV was found whereby those rated highly-irritable showed higher parasympathetic activity throughout the PST; again this finding lost significance after controlling for the effects of anxiety. Irritability was not related to sympathetic activity during the stress test. No effects of self-reported irritability or anxiety on HR or HRV stress responsiveness were found.

Overall, boys with hfASD showed reduced physiological responsiveness to stress compared to TD boys. While anxiety and irritability both influenced physiological stress reactivity in boys with hfASD, irritability contributed to blunted cortisol responsiveness independently of anxiety.

6.1.2 Chapter 4 – Reward processing

This chapter aimed to disentangle the relative effects of anxiety symptoms and ASD traits during reward processing in a large community sample, whilst also taking into account any possible effects of irritability and depression. While reward processing was previously investigated in TD youth with anxiety and in young people with ASD without comorbidities, its relationship with ASD and comorbid anxiety in young people was not previously studied. This is a key question given that close to 40% of ASD youth suffer from comorbid anxiety (Simonoff et al., 2008) and the underlying mechanisms of this comorbidity remain understudied. The main objective of this chapter was to examine whether comorbid ASD traits and anxiety is associated with distinct neurophysiological mechanisms of reward processing (which could represent a case of independent nosology), or whether ASD_{ANX} shares the neural correlates of reward processing seen with ASD traits and anxiety separately. We also employed a longitudinal design to investigate whether brain responses during reward processing underlie the successive comorbidity between ASD traits and anxiety. Finally, we tested whether irritability symptoms were related to neural activation patterns following negative reward feedback, as found previously in young people with SMD (Deveney et al., 2013).

ASD traits. Participants with high compared to low ASD traits displayed reduced BOLD signal in dorsal PFC during reward anticipation and in right medial PFC during negative feedback. These effects remained largely unchanged after accounting for depression and ODD symptoms. Youth with high ASD traits also showed increased activation in the thalamus and putamen following positive feedback, although this effect lost significance after controlling for symptoms of depression.

Anxiety. Participants with high levels of anxiety symptoms showed increased activation in lateral prefrontal regions (right MFG, right IFG) during reward anticipation but reduced activation in these regions following negative feedback (extending to the medial PFC). The results remained significant after controlling for the effects of ODD and depression.

ASD traits x Anxiety. Our interaction analyses revealed that participants high on both ASD traits and anxiety displayed a hyperactivation in the right insula during reward anticipation, and reduced activation in the right-sided caudate, putamen, medial and lateral PFC during negative feedback. The results remained significant after controlling for the effects of ODD and depression.

Longitudinal findings. In participants with high but not low ASD traits, increased right medFG and dorsal cingulate activations during reward anticipation were associated with increased likelihood of anxiety symptoms two years later. The prediction was significant after controlling for baseline anxiety, suggesting that brain activation patterns during reward anticipation can predict new onset of anxiety in those with high ASD traits.

Irritability. Contrary to our hypothesis, irritability symptoms were not associated with changes in brain activation patterns following negative reward feedback in our sample.

Overall, our results suggest that both shared and unique pathophysiological mechanisms may characterise the comorbidity between ASD traits and anxiety. Shared mechanisms included reduced activation in medial prefrontal regions following negative reward feedback, displayed by both the comorbid ASD_{ANX} group and each ASD traits and anxiety separately. The unique insular hyperactivation found in our comorbid group during reward anticipation could represent a distinct pathophysiological process underlying ASD_{ANX}. Furthermore, our longitudinal findings suggest that hyperactivity in the dACC and medFG in the context of reward anticipation may represent a distinct risk factor for the development of anxiety in young people with ASD traits.

6.1.3 Chapter 5 – Alternative measurement

The last empirical chapter explored novel methods of measuring mood states in young people, as a first step towards developing a neuroimaging tool that would overcome the limitations of self-report in youth with ASD. Twenty-one healthy young people completed ASL scanning after neutral, sad, and happy mood states were explicitly induced. We first examined whether ASL is sensitive to detecting rCBF patterns associated with different mood states. Second, we used pattern recognition techniques to test whether the different mood states can be differentiated from each other based on rCBF patterns alone.

Behaviourally, mood induction procedures led to robust changes in self-reported mood ratings. This was accompanied by changes in rCBF patterns.

Sad mood. Sad mood was associated with increased rCBF in the left middle and medial frontal gyri, and decreased rCBF in the right MFG, compared to neutral. We did *not* find the hypothesised increased perfusion in the sgACC following sad mood induction.

Happy mood. Compared to neutral, happy mood was associated with increased rCBF in bilateral medial frontal and cingulate gyri (whole-brain level), amygdala ($p_{FWE\text{-}SVC}$=.051), ventral striatum (as per the hypothesis), and sgACC (ROIs). The effects of happy mood on rCBF patterns were more robust than the effects of sad mood.

Effects of depressive symptoms. Although all participants scored in the non-depressed range, higher levels of depressive symptoms were associated with lower rCBF in the cerebellum and lingual gyrus following both sad and happy mood inductions.

Effects of irritability. Higher levels of irritability were associated with increased perfusion in the right hippocampus (whole-brain level) and right amygdala (ROI) after sad mood induction. Following happy mood induction, there was a trend negative correlation between irritability severity and rCBF in the ventral striatum (ROI; $p_{FWE\text{-}SVC}$=.053).

Pattern recognition. Sad and happy mood states were successfully distinguished from neutral based on their rCBF patterns. However, sad and happy mood states were not differentiated from one another.

Overall, our results suggest that ASL is sensitive to experimentally-induced mood changes in healthy young people, but more subtle rCBF differences may underlie sad mood state as compared to the happy, at least in healthy youth.

6.2 General discussion

6.2.1 Implications for comorbidity models

As discussed in the introduction, thinking about comorbidity models can help understand why there is a strong overlap between emotional symptoms such as anxiety or irritability and ASD. Doing so could help not only with understanding the aetiological mechanism involved, but also inform nosological classification and treatment approaches (Angold et al., 1999; Banaschewski et al., 2007; Caron & Rutter, 1991).

Comorbidity rates are high in young people with ASD, with 70% meeting criteria for at least one additional disorder in a population-based study (Simonoff et al., 2008). As reviewed in Sections 1.2.1.3 and 1.2.2.2, close to 40% of ASD youth also suffer from anxiety, and as many as 20-65% display high levels of irritability symptoms. Despite these high prevalence estimates, research into the mechanisms underlying comorbidity in ASD has been scarce. This is in contrast to other areas of child psychiatry where, for example, both shared and unique aetiological pathways have been identified for comorbid ADHD and tic disorder (Banaschewski et al., 2007).

This thesis aimed to address the gaps in existing literature on comorbidity between ASD and emotional problems in young people. We focused on reward processing and physiological stress responsiveness as possible mechanisms. Previous studies on reward processing in youth with ASD excluded participants with comorbidities and hence may not have been representative of the ASD youth population. In the case of physiological stress responsiveness, the possibly confounding effects of comorbid psychopathology were rarely investigated.

6.2.1.1 Reward processing

The main strengths of our reward study were a large community sample and a 2x2 design that allowed us to investigate the main effects of ASD traits and anxiety as well as their interactions. In the individual discussion section for the reward processing study (Section 4.5), I have discussed how our results fit with various models of comorbidity. To avoid repetition, the reader is directed to that section for a study-specific discussion. Here, I revisit the issue of comorbidity models by discussing our results within a broader perspective, linking our results with existing theoretical accounts of anxiety in the TD population.

Our first main finding with implications for comorbidity models was that activation patterns in the medial PFC appeared to be a common mechanism across ASD traits, anxiety symptoms, and combined ASD_{ANX}. Medial PFC was hypoactivated in high vs. low ASD traits following negative feedback, similar hypoactivation was also found in those with high vs. low anxiety symptoms. However, we also found an interaction whereby those with combined ASD traits and anxiety showed the least medial (as well as lateral) PFC activation following negative feedback compared to other groups. This suggests that while the underlying pathophysiological mechanisms may be shared across ASD and anxiety, combined ASD and anxiety appears to be underpinned by a relatively intensified medial PFC hypoactivation. A reduction in medial PFC activation following negative feedback is commonly found in healthy controls, consistent with this region's role in tracking and updating the reward value of stimuli (Knutson, Fong, et al., 2001; Knutson et al., 2003). Our results suggest a particularly enhanced sensitivity to negative outcomes in those with combined ASD and anxiety. It could be that disrupted reward outcome processing may underlie the co-occurrence of the two conditions. Evidence in favour of this view comes from a study where the hypoactivation in medial PFC in boys with ASD during reward learning (suggesting impaired reward value encoding) was upregulated to healthy control levels with fluoxetine, an SSRI used in the treatment of anxiety (Chantiluke et al., 2015). It is worth mentioning here a seemingly inconsistent finding in TD youth where enhanced, rather than reduced, medial PFC activation after negative reward feedback was associated with behavioural inhibition (Helfinstein et al., 2011). However, the behaviourally-inhibited temperament was based on historical tendencies and the historically inhibited vs. non-inhibited participants did not differ on anxiety at the time of scanning (Helfinstein et al., 2011), unlike participants in our sample.

Pending replication, two main results from the reward anticipation condition suggested that both cross-sectional and successive comorbidity between ASD traits and anxiety may be underpinned by distinct neural correlates to those found for anxiety in participants low on ASD traits. First, we found a distinct pattern of right insula hyperactivation in the ASD_{ANX} and ASD_{EMO} groups during reward anticipation. Second, increased activations in the right medFG and right dACC during anticipation predicted new-onset of anxiety at two-year follow up in those with high but not low ASD traits.

Hyperactivation in the insula has been previously linked to anxiety in the TD population; e.g. insula activation increased in response to symptom provocation across a range of anxiety disorders (Lorberbaum et al., 2004; Mataix-Cols et al., 2004; Rauch, Savage, Alpert, Fischman, & Jenike, 1997), and insula hyperactivation was reportedly reduced with SSRI treatment in adults with GAD (Hoehn-Saric, Schlund, & Wong, 2004). Paulus and Stein (2006) proposed that aberrant interoceptive prediction signalling may underlie the relationship between insular hyperactivation and anxiety. According to their theoretical framework, anxious people are hypersensitive to detecting the difference between their expected and perceived body state in certain contexts. This leads to the anticipation of negative outcomes, consistent with insular involvement in predicting the salience of aversive events (Chua et al., 1999; Loewenstein et al., 2001; Ploghaus et al., 1999; Wittmann et al., 2014), and triggers anxious symptomatology such as worrying thoughts and behavioural avoidance (Paulus & Stein, 2006). Several factors could explain why those with ASD may be particularly susceptible to aberrant interoceptive prediction signalling, including atypical interoceptive awareness (Cascio et al., 2012; Fiene & Brownlow, 2015; Garfinkel et al., 2016; Santos et al., 2011), sensory over-responsivity (S. A. Green et al., 2012), and altered physiological responsiveness to stress (central to Chapter 3 in this thesis). Importantly, a recent study showed that while adults with ASD report heightened sensitivity to internal sensations, they are less accurate than TD controls in correctly identifying the pace of their heartbeats (Garfinkel et al., 2016), consistent with the increased likelihood of interoceptive prediction errors in ASD. Previous literature and our results therefore suggest that hyperactivity in the insula may be particularly pronounced in those with combined ASD traits and anxiety. Insular hyperactivity and atypical interoceptive processing may render these individuals particularly susceptible to anticipating negative outcomes, even in an otherwise non-threatening context. For instance, they may have a heightened concern of responding incorrectly to the upcoming target and failing to obtain a reward.

The pattern of distinct neural correlates of reward processing held longitudinally, so that increased medFG and dACC activations predicted future anxiety in those with high but not low ASD traits. This result may be potentially linked with our insula finding, since both the medFG (Bruguier, Preuschoff, Quartz, & Bossaerts, 2008) and the dACC (Botvinick, Braver, Barch, Carter, & Cohen, 2001; Botvinick, Cohen, & Carter, 2004; Carter, Botvinick, & Cohen, 1999) receive input from the insula. The role of the medFG during reward anticipation has been proposed to involve monitoring of contextual factors such as the probability of reward (Knutson, Taylor, Kaufman, Peterson, & Glover, 2005) and decision making under conditions of risky choice (Preuschoff, Bossaerts, & Quartz, 2006), whereas the dACC is thought to have an integral role in error and conflict monitoring (Botvinick et al., 2001; Botvinick et al., 2004). It is possible that anticipatory anxiety (underpinned by insula hyperactivation) may create a context where enhanced conflict monitoring is required to maintain on-task attention and motivation when faced with competing effects of worrisome thoughts and/or avoidant tendencies. This premise is central

to the Compensatory Error Monitoring Hypothesis by Moser et al (2013). Based on meta-analytic evidence from ERP studies on anxiety and response conflict, Moser and colleagues proposed that increased dACC activation is needed in order to compensate for the distracting effects of 'anxious apprehension' when performing otherwise non-affective tasks, consistent with Eysenck's Attentional Control Theory (Eysenck, Derakshan, Santos, & Calvo, 2007). Similar compensatory mechanism may underlie the hyperactivation in the medFG, especially as anxiety in TD children has been proposed to induce maladaptive, 'risk-aversive' decision making by reducing the expected positive value of outcomes (Sonuga-Barke et al., 2016). Emerging evidence for the joint role of medFG and dACC in anticipatory anxiety comes from a PET study where healthy adults anticipated an electric shock (Simpson, Drevets, Snyder, Gusnard, & Raichle, 2001). While the least anxious participants showed rCBF reductions in medFG and dACC during anticipation, no reduction and a slight increase in rCBF was reported for highly-anxious participants. Our results suggest that those with combined anxiety symptoms and high ASD traits may be even more susceptible to an increased medFG and dACC response during anticipatory anxiety. However, we did not test whether our participants found the anticipation phase of the reward task anxiety-provoking; future studies may benefit from including a self-reported measure of anticipatory anxiety in order to determine its role in generating heightened insula, medFG and dACC activations. Evidence in support of our model also comes from a study where children with ASD allocated disproportionately more attentional resources to interoceptive, rather than exteroceptive, sensory cues during the rubber hand illusion paradigm (Schauder, Mash, Bryant, & Cascio, 2015). Overall, the comorbid ASD_{ANX} group in our study appears to show significantly enhanced anxiety-related brain activation patterns during reward processing.

6.2.1.2 Stress reactivity

Similarly to studies on reward processing, the question of comorbidity has been largely neglected in physiological stress responsiveness research. Although Groden et al (1994) suggested that irritability may be related to anxiety and the way young people with ASD respond to stress, our study was the first to test this experimentally. In TD youth, most studies that investigated physiological reactivity to stress in externalising disorders failed to assess co-occurring internalising problems (Alink et al., 2008). This is surprising as the two may be associated with distinct biological profiles (see Section 2.3.3.2) and psychosocial stress tests have been shown to induce states of fear and anxiety as well as anger in TD adults (Lupis et al., 2014; Moons et al., 2010). Also in our study, while anxiety was the most commonly reported emotion associated with the stress test, roughly a quarter of participants (both ASD and TD boys) reported "feeling annoyed" during the task. This suggests that both anxiety and irritability are important to consider when assessing physiological responses to stress.

Before moving on to discussing the effects of irritability and anxiety on stress responsiveness in our study, a limitation needs to be mentioned. Since all TD participants in our sample displayed low levels of irritability and the range of irritability scores was low within this group, we were unable to implement a 2x2 design similar to the one used in our reward processing study. This was due to the irritability measure being introduced into the study at a later date, with the primary outcome being anxiety (Hollocks et al., 2014). Future studies should address this limitation by recruiting a matched TD group that would show comparable variance in irritability scores to participants with ASD. With this caveat in mind, we can compare the effects of irritability on stress responsiveness in our ASD sample with that of TD adolescents studied in existing literature. While irritability has not been studied directly in physiological stress response studies in TD youth, evidence exists for the effects of more broadly defined externalising symptoms.

Cortisol stress responsiveness. Similarly to previous TSST studies in young people, we found a relatively dampened cortisol response to stress in the ASD vs. TD sample (Corbett et al., 2012; Hollocks et al., 2014; Lanni et al., 2012; Levine et al., 2012), even despite a statistically equal increase in subjective stress in both groups. Only two previous studies investigated the effects of comorbid emotional symptoms (specifically, anxiety) on psychosocial stress responsiveness in young people with ASD. Lanni et al (2012) found no effects of anxiety on cortisol stress-response, while Hollocks et al (2014) reported that participants with comorbid ASD and anxiety displayed the lowest cortisol reactivity to stress compared to both TD controls and those with ASD but without anxiety. Both findings differ from the reported cortisol hyper-responsiveness to stress in TD children with social phobia (Faravelli et al., 2012), as well as the general finding that fear of negative social evaluation reliably induces cortisol release in healthy controls (Dickerson & Kemeny, 2004). This suggests that anxiety may be associated with different underlying physiological mechanisms in ASD compared to TD youth, akin to our reward processing findings. However, our current study extends these findings and suggests that co-occurring irritability may influence cortisol stress responsiveness independently of the effects of anxiety. Few studies in TD youth have investigated the relative effects of externalising and internalising symptoms on cortisol stress reactivity. Van Goozen and colleagues reported that while children with ODD/CD showed a dampened cortisol response to stress relative to HC, the dampening was strongest in those high on externalising symptoms but low on anxiety (van Goozen et al., 2000; van Goozen et al., 1998). Consistent with these results, we found that while the association between irritability and dampened physiological stress reactivity remained significant after controlling for anxiety, its effect size decreased slightly, suggesting a possible interplay between irritability and anxiety in youth with ASD. Nevertheless, due to paucity of existing evidence in the field across TD and ASD youth, and the fact that (parent-reported) irritability and anxiety were moderately correlated in our study, more research is needed to make

firm conclusions about the relative effects of irritability and anxiety on cortisol stress responsiveness.

ANS stress responsiveness. Consistent with previous psychosocial stress studies, HR stress reactivity was significantly dampened in our ASD sample relative to TD controls (Hollocks et al., 2014; Jansen et al., 2003; Kushki et al., 2014). Furthermore, the dampening effect of irritability on HR responsiveness lost significance after controlling for anxiety, consistent with previous psychosocial stress studies in ASD youth where anxiety was associated with decreased HR stress-response (Hollocks et al., 2014; Kushki et al., 2014) but see (Jansen et al., 2003; Klusek et al., 2013). This is different to HR reactivity in TD children with social phobia, who tend to show overall elevated mean HR throughout the stress test, but comparable HR stress responsiveness relative to healthy controls (Kramer et al., 2012; Schmitz et al., 2011). Again, this suggests that the pathophysiological mechanisms of the comorbidity between ASD and anxiety may differ to how anxiety presents in the TD population.

With regards to HRV, while we did find a main effect of parent-reported irritability so that those rated highly-irritable displayed increased parasympathetic activity before, during, and post-stressor, irritability did not affect sympathetic or parasympathetic stress *reactivity*. Previous studies in TD children with externalising problems found reduced stress reactivity in both ANS branches – see Section 2.3.3.2 and meta-analyses on this topic (Graziano & Derefinko, 2013; Lorber, 2004) for a review. The lack of such association in our ASD sample could suggest that different pathophysiological mechanisms may underlie such problems in TD vs. ASD youth. However, alternative explanations are possible. First, the effects of externalising problems on ANS stress reactivity in TD youth were small, and comparable to the effects of internalising symptoms, akin to the moderate correlation between parent-reported irritability and anxiety in our sample, making the relative effects of irritability and anxiety difficult to disentangle. Second, while our study used a scale developed specifically to measure irritability, psychosocial stress studies in TD youth conceptualised externalising symptoms more broadly, including acts of aggression and conduct problems. As previously discussed, in TD youth, such symptoms were shown to differ from irritability in their cross-sectional and longitudinal correlates (Stringaris et al., 2009; Stringaris & Goodman, 2009a, 2009b) as well as genetic underpinnings (Stringaris, Zavos, et al., 2012). It remains to be tested whether analogous differences between irritability and other externalising symptoms can be found in the domain of physiological responsiveness to stress. Indeed, our finding that the association between irritability, HR and HRV lost significance after controlling for anxiety suggests that irritability and emotional problems may be closely linked during ANS stress response in boys with ASD. This is consistent with the previously-reported cross-sectional and longitudinal link between irritability and emotional problems in ASD youth (Mandy et al., 2014; Simonoff et al., 2012) and is a more general feature of shared mechanisms underlying emotional expression (Stringaris, 2015).

6.2.1.3 Comparative summary and directions for future research

Overall, both our studies suggest that there may be neurophysiological differences between how anxiety presents in youth with vs. without ASD symptomatology. Participants with ASD and anxiety appear to display both distinctive neurophysiological patterns (e.g. hyperactivation in the insula, medFG and dACC during reward anticipation; reduced physiological stress responsiveness) as well as enhanced responses similar to those seen in the main effect of anxiety (e.g. further decrease in medFG following negative reward feedback). Our findings with irritability are less clear-cut, and suggest that while the effects of anxiety on reward processing remain significant after controlling for ODD symptoms (and irritability does not affect neural responses following negative feedback), cortisol hypo-responsiveness to stress in ASD may be driven by the co-occurring irritability.

Several factors pertaining to both studies limit conclusions that can be drawn about the model of comorbidity underlying ASD and emotional problems in young people. First, in either study, it was impossible to establish which specific anxiety disorders (for example, GAD or social anxiety) influenced neurophysiological response patterns (Guyer, Choate, Detloff, et al., 2012; Kessel et al., 2015). Second, specific effects of "pure", non-comorbid irritability, anxiety, and ASD on reward processing and stress responsiveness have not yet been sufficiently established in the literature. This limits comparisons that can be made with cases where these conditions are comorbid. Few studies have used the recommended 2x2 design (Banaschewski et al., 2007) that would allow disentangling the relative influence of each symptom on pathophysiological mechanisms. In addition, irritability was often assessed as part of a wider spectrum of externalising problems, rather than using specific irritability scales. In terms of what we know about the effects of "pure" ASD, while reward processing studies in ASD youth excluded participants with comorbidities, this was not the case in studies of physiological stress responsiveness.

Ideally, future studies should employ multimodal assessment methods so that the effects of ASD and emotional problems can be evaluated at several levels of explanation within the same sample. Combining behavioural, physiological, and neuroimaging approaches may be particularly effective. First, employing self-reported state (on-task) measures would allow us to link neurophysiological response patterns to individual differences in experience that may give rise to these responses. This would, for example, inform our hypothesis that hyperactivation in the insula may be associated with aberrant interoceptive predictions during anticipation, leading to the need for more dACC involvement in conflict monitoring – bringing implications for targeted treatment of comorbid ASD and anxiety. While reporting on internal states may be problematic for some youth with ASD (see Section 1.2.3), paradigms such as the heartbeat perception task (Schandry, 1981), that rely on strictly physical cues, have been used successfully in ASD (Garfinkel et al., 2016; Schauder et al., 2015). Second, combining methodologies is essential to investigate the

possible top-down cognitive control effects on physiological stress response patterns. Several theoretical frameworks have noted the possible role of prefrontal regions in regulating physiological stress responses via contextual appraisal in young people with ASD (Corbett et al., 2012; South et al., 2011), yet this hypothesis has not yet been tested experimentally. In TD children, there is meta-analytic evidence for the association between adaptive physiological stress responsiveness (parasympathetic withdrawal under challenging conditions) and better cognitive functioning, as well as lower levels of psychopathology (Graziano & Derefinko, 2013). This is not surprising considering that deficient cognitive control is seen as a maintaining factor in anxiety disorders (Bishop, Duncan, Brett, & Lawrence, 2004; Eysenck et al., 2007). A similar aberrant cognitive control mechanism could underlie mood and anxiety problems in ASD and be associated with dysregulated stress responses. Future research will be essential to test this hypothesis, as well as to uncover the neural networks underlying such cognitive control dysregulation in ASD youth. A recent meta-analysis of fMRI studies on response inhibition (a function that relies on effective cognitive control) concluded that individuals with ASD display both shared and distinct brain activation patterns relative to healthy controls and patients with OCD (Carlisi, Radua, Mataix-Cols, & Rubia, under review). The two ASD-specific mechanisms found during response inhibition processing were reduced dlPFC and increased PCC activations. Both these activation patterns were displayed by the comorbid groups (ASD_{ANX} and ASD_{EMOT}) in our reward study: increased PCC activation when anticipating reward in 'core' ASD_{EMOT} vs. ASD_{ONLY} and reduced dlPFC activation following negative feedback in ASD_{ANX} and ASD_{EMOT} vs. ASD_{ONLY}. This again suggests that some neural correlates underlying comorbid ASD and anxiety may be different to how anxiety presents in the TD population, a possible case of "true anxiety phenotypically altered by ASD pathogenic processes" (Wood & Gadow, 2010).

In Figure 6.2, I provide a schematic summary of the proposed relationships between key findings discussed in this section. It is worth noting that the quality of affective responses and emotional difficulties in young people with ASD may be additionally shaped by contextual factors. First, theoretical accounts based on animal models propose that the choice between a "fight" or "flight" response may depend on environmental contingencies, e.g. whether the option to escape a stressful situation is available (Rolls, 2007). Stringaris and Taylor (2015) suggested that similar contextual effects may apply to emotional responses in human irritability, whereby subjectively inescapable contexts may lead to anxious withdrawal and depressive hopelessness, whereas an opportunity of escape may increase the likelihood of reactive aggression. In this thesis, we did not find an association between reward processing and irritability, suggesting that our task may have been mostly anxiety-inducing (perhaps due to the absence of a frustrative reward loss condition). In contrast, the participants' responses following psychosocial stress induction revealed both irritability and anxiety as the main feelings experienced during the task. A second contextual factor could be the young person's previous experience. Novel or unpredictable situations may be particularly anxiety-inducing due to the associated uncertainty. By contrast,

positive reinforcement of an angry reaction (e.g., parents relaxing a bedtime rule following a temper tantrum) may increase the likelihood of a child reacting angrily in a similar context. While they may prove difficult to control, contextual factors and individual differences in experience should be taken into account when designing studies to test the mechanisms of comorbidity between ASD and emotional problems. This may, for example, help disentangle the effects of anxiety and irritability during emotional processing and stress response.

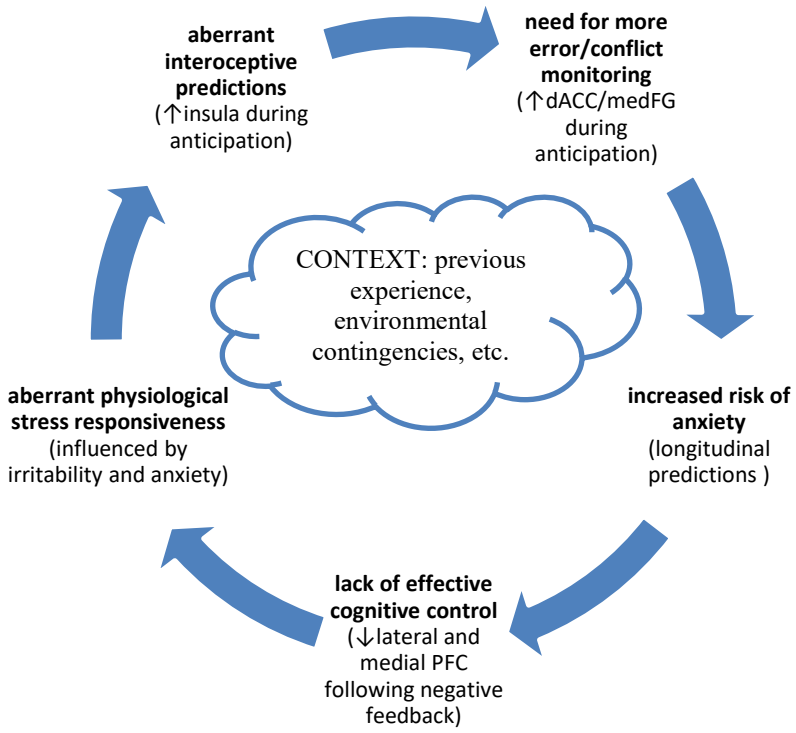


Figure 6.2. Hypothetical model explaining the co-occurrence of ASD and emotional difficulties based on key results from this thesis and the extant literature. Young people with ASD may be particularly susceptible to anticipatory anxiety that is associated with insular hyperactivity in an otherwise non-threatening context of reward anticipation. Additional attentional resources and enhanced conflict monitoring by the dACC may be required to meet task demands when faced with the competing stressors. Hyperactivity of attentional brain networks further increases the risk of anxiety in ASD, as evidenced by our longitudinal findings. Youth with ASD and anxiety may show ineffective cognitive control in stressful situations, such as negative feedback. Ineffective cognitive control may lead to dysregulation of the physiological mechanisms underlying stress response, such as reduced HPA axis and ANS stress reactivity seen in our ASD sample. Aberrant physiological stress responsiveness may further contribute to impaired interoceptive predictions and insula hyperactivation. Contextual factors such as previous experiences and environmental contingencies (e.g., possibility of escaping the stressful situation) may modify the quality of emotional response, resulting in symptoms of anxiety, irritability, or depression. See main text for a discussion.

ANS, autonomic nervous system. *dACC*, dorsal anterior cingulate cortex. *HPA*, hypothalamic–pituitary–adrenal. *medFG*, medial frontal gyrus. *PFC*, prefrontal cortex.

6.2.2 Implications for measurement

As mentioned in Section 1.2.3, traditional methods of assessing emotional symptoms are problematic in the ASD population. Many scales used to assess mood problems in TD children have not been validated in the ASD population; and while evidence suggests that children with high-functioning ASD may report reliably on some symptoms, including anxiety (Van Steensel, Deutschman, et al., 2013) and the negative outcomes of their social interaction difficulties (Knott et al., 2006), self-report validity may be particularly limited in those with lower IQ.

In this thesis we used both traditional and alternative methods of assessing mood. The study in Chapter 3 assessed mood in a traditional manner, collecting self- and parent-reports of irritability with the ARI questionnaire in a hfASD sample (Chapter 3). The other study (Chapter 5) explored alternative, neuroimaging methodologies, with a long-term goal of developing a measure of mood unaffected by reporting difficulties. Implications and future directions for both assessment methods are presented below.

6.2.2.1 Self and parent report

Our results suggest that boys with hfASD report reliably on their irritability and that the pattern of their irritability symptoms closely resembles that of TD boys with severe, chronic irritability (SMD) while being clearly differentiated from healthy controls, as is the case in TD youth (Stringaris, Goodman, et al., 2012). These results are consistent with, and extend, previous findings where more broadly-defined (and solely parent/teacher reported) irritability symptoms in ASD youth showed comparable characteristics to those reported by TD youth with ODD/SMD (Gadow et al., 2008; Simonoff et al., 2012). Also consistent with TD literature (Stringaris, Goodman, et al., 2012), parent- and self-reports of irritability were strongly correlated in our hfASD sample, also in terms of the associated functional impairment.

Apart from replicating our findings in an independent sample, future studies should examine the validity of irritability reporting in hfASD, which we have not addressed. For instance, convergent validity of self-reported irritability as measured by the ARI could be compared with the gold standard clinical interview. While an interview schedule specific to the newly-coined DSM-V DMDD category is not yet available, the K-SADS supplement developed for diagnosing SMD could be used (Kaufman et al., 1997; Leibenluft et al., 2003). In addition, once scales developed for the purpose of measuring irritability are validated in ASD youth, future studies should examine whether similar cross-sectional and longitudinal associations exist between irritability and other psychopathology across ASD and TD youth. Existing evidence from studies that used irritability subscales derived from pre-existing measures suggest that irritability symptoms in ASD are indeed selectively associated with internalising, rather than externalising

problems (Mandy et al., 2014; Simonoff et al., 2012), consistent with findings in TD youth (Stringaris et al., 2009; Stringaris & Goodman, 2009a, 2009b).

6.2.2.2 Alternative measurement methods

While our pilot study suggests that ASL is sensitive to detecting rCBF changes associated with mood states when compared to a neutral condition, whole-brain perfusion patterns alone were not sufficient to differentiate between sad and happy mood states. This is somewhat inconsistent with previous reports suggesting that out of different emotions, those of opposite valence (pleasant vs. unpleasant) and opposite arousal patterns (high vs. low), such as happiness and sadness, respectively, are most likely to be distinguished based on both subjective experience and associated physiological patterns (Lench, Flores, & Bench, 2011; Lindquist, Siegel, Quigley, & Barrett, 2013). One possibility is that mood induction experiments that measure longer-lasting *mood states* are inherently more likely to produce higher rates of between-subjects variability, as opposed to event-related paradigms that elicit more discrete, short-term *emotions*. As a result, neural responses to sad and happy mood induction may have not elicited sufficiently uniform and distinct rCBF patterns in order to be accurately distinguished from one another. Second, the amount of rCBF change in response to mood induction may depend on previous emotional experience. For example, a PET study revealed rCBF differences in response to sad mood induction in healthy adults with vs. without a history of depression, whereby remitted adults with a history of depression displayed a unique decrease in rCBF in the medial OFC (Liotti et al., 2002). For the purposes of our pilot methodological study, we specifically recruited young people with no personal or family history of low mood, and with low current levels of emotional symptoms. Although this permitted a more controlled experimental set-up in which to test the effectiveness of ASL in measuring absolute rCBF changes associated with induced mood states, healthy young people may not have been equally susceptible to the effects of sad and happy mood inductions. A future ASL mood induction study in young people with depression will be needed to test this possibility. It is important to note that in investigating the possible underlying markers of mood states we are not necessarily implying a traditionally 'locationist' view whereby distinct moods can be distinctly localised to particular regions in the brain. Existing meta-analytic evidence suggests that more general, distributed brain networks are likely to generate different types of emotional experience (Lindquist, Wager, Kober, Bliss-Moreau, & Barrett, 2012). This suggests that connectivity methodologies may be useful in identifying how different mood states affect the inter-regional relationships within brain networks. ASL shows promising results in resting-state functional connectivity studies in adults (Chuang et al., 2008; Dai, Varma, Scheidegger, & Alsop, 2016) and could bring important insights into how different regions interact to produce rCBF patterns associated with mood states.

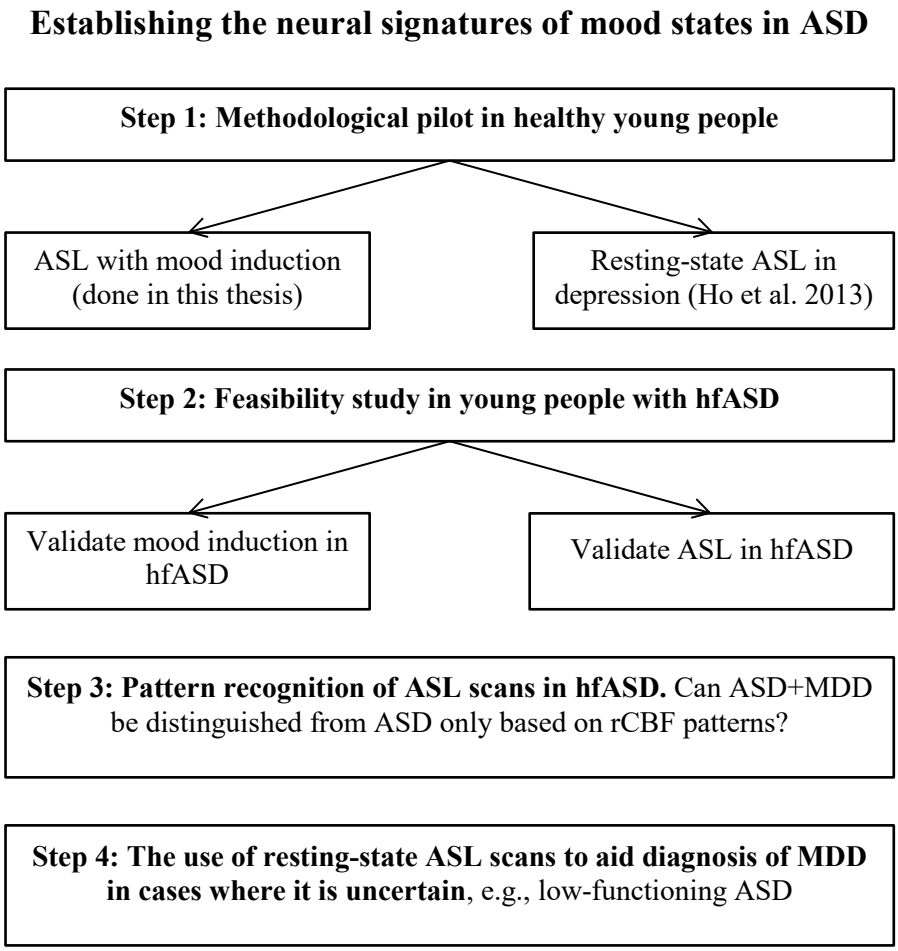
The main challenge when attempting to extend this research into the ASD population is likely to be the feasibility of inducing mood states in young people with ASD. On one hand, some forms of emotional processing appear preserved in ASD youth. For instance, we know from empathy research that young people with hfASD display similar affective empathy to healthy controls, and only struggle on empathy tasks when cognitive perspective-taking is required (Bird & Viding, 2014; Jones, Happé, Gilbert, Burnett, & Viding, 2010; Lockwood, Bird, Bridge, & Viding, 2013; Rogers et al., 2006; Schwenck et al., 2012). In addition, this thesis demonstrated equal levels of self-reported distress in hfASD and TD boys following psychosocial stress induction. On the other hand, research into mood elaboration and mental imagery in ASD – both likely to be required during mood induction paradigms – has been scarce. Evidence from fMRI studies suggests that while adults with hfASD and TD adults show similar brain activation patterns when self-reporting on the valence (pleasantness/unpleasantness) of picture stimuli (Silani et al., 2008; Tseng et al., 2016), group differences emerge when comparing the neural mechanisms of mood elaboration (Silani et al., 2008). An alternative way of investigating rCBF patterns associated with mood states in ASD youth would be to compare resting-state brain activation patterns in those with vs. without co-occurring depression. General disadvantages of this approach were discussed in Section 0 and concern between-patients heterogeneity. A further challenge pertains to ascertaining depression in individuals with ASD (see Section 1.2.3), and the fact that depression-specific rCBF changes would need to be distinguished from those occurring as a result of ASD in its own right (Burroni et al., 2008; Gupta & Ratnam, 2009). Studies may benefit from recruiting young people with hfASD and low levels of alexithymia in the first instance, where the likelihood of incorrectly diagnosing depression via clinical interview is likely to be relatively low. Overall, several challenges pertain to the research on rCBF patterns associated with mood states in young people with ASD. The avenues for future research are summarised in Figure 6.3 (page 207) and should be considered in light of the limitations discussed here. Ultimately, if reliable signatures of mood states can be established in the ASD population, a crucial research aim would be to test whether those with comorbid ASD and depression can be distinguished from youth with ASD without depression based on rCBF patterns alone.

Finally, while discussing alternative measurement methods it is worth noting that some have suggested using physiological activation levels, such as the cortisol awakening response (CAR), as validation markers for self-reported anxiety in ASD (Bitsika & Sharpley, 2015; Bitsika, Sharpley, Sweeney, & McFarlane, 2014). However, there is currently insufficient evidence in support of this view, and several factors argue against using cortisol levels as a marker of anxiety in youth with ASD. First, considerable within-person variability in morning cortisol levels over time as well as between-subjects differences in circadian rhythms have been reported in children with ASD (Corbett, Mendoza, Abdullah, Wegelin, & Levine, 2006; Corbett, Mendoza, Wegelin, Carmean, & Levine, 2008), limiting the reliability of cortisol markers. Inconsistent CAR results have been reported, with some studies reporting absence of CAR in children with ASD (Brosnan,

Turner-Cobb, Munro-Naan, & Jessop, 2009) and some finding no difference in CAR between ASD and TD youth (Zinke, Fries, Kliegel, Kirschbaum, & Dettenborn, 2010). In addition, cortisol levels may be affected by medication use, although the same limitation concerns the use of potential fMRI markers of mood states. Lastly, previous findings in TD youth and our results suggest that irritability as well as anxiety may influence physiological responsiveness to stress, and that these often co-occur in youth with ASD, possibly limiting the specificity of cortisol levels as indicative of anxiety.

With these caveats in mind, future research into markers of anxiety and mood states that would be unaffected by self- and observer-reported limitations could potentially improve diagnostic accuracy via developing adjunct, or alternative assessment methods. A recent pilot conducted in a subset of ASD and TD participants from the physiological reactivity study presented in this thesis showed that non-verbal, computerised facial coding metrics can predict stress-induced cortisol changes over and above parent-reported anxiety (Kaurin et al., submitted). This suggests an added value of automated assessment tools, in this case acting as an intermediate step between purely physiologically-based and purely informant-based measures.

Figure 6.3. Future directions for research on the neural correlates of mood states in young people with ASD. Future research efforts should be considered in light of limitations discussed in the main text.



ASL, arterial spin labelling. *(hf)ASD*, (high-functioning) autism spectrum disorder. *MDD*, major depressive disorder. *rCBF*, regional cerebral blood flow.

6.3 Limitations

Specific limitations concerning each study in this thesis were noted at the end of the discussion sections for each individual chapter. Here, I revisit these limitations while also taking a broader approach to discuss limitations that are more general or are relevant to the thesis as a whole.

I also noted several limitations in the previous section of this chapter (Section 6.2), where I suggested how future studies may address them and test our predictions about the mechanisms underlying comorbidity between ASD and emotional problems in youth. These limitations included: (1) lack of sufficient variance in irritability scores among TD participants in our stress reactivity study; (2) the need for a multi-method study that would examine the mechanisms

underlying comorbidities in ASD at several levels of explanation within the same sample; (3) the need to study the validity of irritability reporting by young people with ASD; (4) feasibility of ASL mood induction research in ASD.

6.3.1 Different samples

Each empirical chapter in this thesis used a separate sample, preventing direct comparisons across the investigated modalities from being made.

An important difference across samples was the characterisation of ASD. In Chapter 3 we used a clinic-based group of boys with a diagnosis of ASD. Although the diagnosis was not confirmed with the gold-standard ADI or ADOS in all cases, we took steps to limit the possibility of false positives by requiring at least a clinical diagnosis of ASD and an SCQ cut-off score of ≥15. In contrast, Chapter 4 focused on reward processing in a community sample where participants were assigned to groups of low vs. high ASD *traits*. While a validated assessment tool (the DAWBA) was used, and ASD traits were based on DSM-IV definitions (current at the time of assessment), the study was not designed to assess reward processing in youth who met the diagnostic threshold for ASD. Nevertheless, we found similar effects of anxiety/emotional symptoms on reward processing in our primary analyses and when ASD traits were defined more strictly, suggesting that the results may generalise across ASD trait severity. In addition, our approach is consistent with the dimensional view of ASD and its underlying aetiological mechanisms (see Section 1.1.4 for a review). Furthermore, by using a community- rather than clinic-based sample we also avoid the risk of referral bias, an important issue when investigating comorbidity (Angold et al., 1999; Caron & Rutter, 1991). Finally, as mentioned above, the methodological study in Chapter 5 used TD healthy controls, and rCBF patterns associated with mood states in young people with ASD remain to be studied. It is currently unclear whether mood induction approaches can be used in young people with ASD, even those who are high-functioning, which limits implications for the measurement of mood states using ASL in the ASD population.

The age of participants also varied across the individual studies, ranging from 10-16 in the physiological reactivity study, through 14 (at time 1) in reward processing, up to 16-18 in the ASL mood induction study. Particular care should be taken when comparing fMRI results across development due to continued brain maturation during adolescence, particularly in the frontal regions (Paus, 2005), and evidence for delayed frontal maturation (Zilbovicius et al., 1995) and longitudinal changes in cortical thickness (Hardan, Libove, Keshavan, Melhem, & Minshew, 2009) in children with ASD.

While males and females were almost equally represented in both our fMRI studies, the physiology study only included boys. Although ASD has higher prevalence rates in males than

females, it remains to be studied whether similar effects of irritability and anxiety on physiological responsiveness are present in girls with ASD. Gender differences seem particularly worth investigating in light of recent findings where associations between stress-induced cortisol responses and anger in adults (Lupis et al., 2014) and between cortisol levels, heart rate, and internalising problems in youth (Hartman et al., 2013) were only found in males.

An analogous point concerns the need to study neurophysiological mechanisms of mood states and stress response across the spectrum of intellectual ability, given that a large proportion of youth with ASD display IQ scores below 70 (Elsabbagh et al., 2012; Grzadzinski et al., 2013), an exclusion criterion in the studies presented in this thesis. While task demands will need to be adapted for lower-functioning youth, developmentally-appropriate stress-inducing tasks have been successfully used in this population (Groden et al., 2005; Willemsen-Swinkels, Bakermans-Kranenburg, Buitelaar, van IJzendoorn, & van Engeland, 2000). Regarding neural signatures of mood states, if reliable rCBF markers can be established in youth with hfASD, a resting-state ASL scan could be sufficient to identify atypical perfusion patterns indicating a possible mood problem.

6.3.2 Experimental design and methodology

The ecological validity of the TSST has been questioned due to its structured design and the use of consistent, concrete social feedback that may not translate to the dynamic nature of psychosocial stressors one is likely to encounter in everyday life (Lanni et al., 2012). Nevertheless, the TSST is currently the most well-established and widely-used psychosocial stress induction paradigm, not least due to its structured nature that allows reproducibility across studies. Its reliability in inducing physiological stress responses made it the paradigm of choice for this thesis. Of note, as mentioned previously, we introduced a change to the original TSST by replacing the mental arithmetic task with the Rey-Osterrieth Complex Figure test (Osterrieth, 1944; Rey, 1941). While this was done to increase the likelihood of participants with hfASD finding the task stress-inducing, care should be taken when making direct comparisons to previous studies that used the original version of the TSST (Kirschbaum et al., 1993).

Second, as mentioned in the individual discussion section for the reward study, our negative feedback condition may have not been sufficiently frustration-inducing to produce brain activation patterns previously reported in children with severe irritability (Deveney et al., 2013). To disentangle the effects of irritability and anxiety during negative reward feedback processing, future studies may benefit from including a loss condition in their MID paradigms, as used by Deveney and colleagues, or asking participants to report on their feelings of anger and anxiety during the task. However, the use of detailed paradigms with several task conditions may not be feasible in studies that use large, population-based samples, recommended when investigating

comorbidity. The same challenge concerns the inclusion of our MID task learning phase in the scanner to investigate the neural mechanisms of stimulus-reward learning. Another limitation of our reward study is the use of a single type of reward (points converted to chocolate treats upon finishing the task). It remains to be tested whether similar neural mechanisms underlie the processing of strictly monetary or social types of reward in youth with ASD traits and anxiety. Indeed, some (Delmonte et al., 2012; McPartland, Crowley, et al., 2012; Stavropoulos & Carver, 2014) but not other studies (Kohls et al., 2013) reported differences in how children with ASD process different types of reward.

Finally, the ASL study in Chapter 5 used a fixed order of mood induction conditions. While used deliberately to maximise the power to detect mood-specific rCBF patterns (randomisation would require a larger sample size), an order effect cannot be ruled out. For example, since we know little about the temporal characteristics of rCBF changes associated with mood states, it could be that the effects of sad mood induction only manifested later, e.g. during the 'happy' mood induction scan. This could explain why we observed hyperactivation in the sgACC during the happy, but not the sad, condition. Another limitation concerns the self-reported measure of mood states. As mentioned in the discussion section in Chapter 5, the responses may have been influenced by the participants' desire to please the examiner. In addition, self-reported anger ratings in response to mood induction were not recorded. Introducing an on-task measure of anger in future studies may shed light on our findings with trait irritability, where highly-irritable participants showed increased rCBF in right-sided hippocampus and amygdala following sad vs. neutral mood induction.

6.3.3 Assessment of psychopathology

An important strength of this thesis is the simultaneous assessment of multiple emotional symptoms in each individual study. In each study, we assessed the effects of anxiety and irritability that tend to co-occur in ASD but may be associated with different neurophysiological profiles. Chapters 4 and 5, but not Chapter 3, also considered depressive symptoms. Nevertheless, a few limitations need to be mentioned.

First, irritability measurement could not be obtained from all participants in Chapter 3 due to being introduced to the study when data collection was already ongoing. Attrition was particularly apparent for self-report, where only 29 out of 52 boys with ASD, and 9 out of 23 TD boys, provided irritability data. Future studies with larger sample sizes are needed to replicate our findings and to enable the comparison of irritability effects on physiological stress responsiveness in ASD vs. TD youth. In addition, we did not assess symptoms of depression, closely linked to irritability (Stringaris & Goodman, 2009a), that may have affected physiological stress responses in their own right.

Second, none of the three studies distinguished between different types of anxiety, although we know from the reward processing literature in TD youth that different anxiety disorders may be associated with distinct neurophysiological correlates (Guyer, Choate, Detloff, et al., 2012; Kessel et al., 2015). In addition, emotional problems were assessed by parent report in the reward study (Chapter 4). While used deliberately to maintain reporting source consistency between ASD traits and emotional problems, it is possible that some emotional symptoms experienced by the participants were missed by their parents.

Third, the chronicity of emotional symptoms was not investigated. Previous studies in TD children have shown that HPA axis reactivity may vary depending on the time passed since the onset of emotional difficulties. Recent-onset symptoms tend to be associated with stress-induced cortisol increases, while more chronic problems are related to a reduction in HPA axis reactivity (Booij et al., 2013; Ruttle et al., 2011). Cortisol responsiveness to stress is also reportedly reduced with increased incidence of stressful life events in children (Armbruster et al., 2012; Jaffee et al., 2015). In addition, animal models suggest that chronic stress may lead to impaired reward-based decision making via reorganisation of the frontostriatal brain networks (Dias-Ferreira et al., 2009). Most of the measures used in this thesis had a six-month reporting timeframe (ARI, SDQ, DAWBA), while the SCAS does not have a set timeframe for symptom reporting. The time passed since the onset of these difficulties was not explicitly assessed. It remains possible that our results may have been influenced by chronicity of emotional problems, particularly in light of cortisol hyporesponsiveness observed in boys with ASD and irritability. Future studies in ASD youth should aim to disentangle the effects of co-occurring emotional symptoms *per se* from the effects of their chronicity, considering that some comorbidities in ASD manifest as early as toddlerhood (Mannion & Leader, 2013). Thorough psychiatric interviews may be advantageous for collecting a detailed history of both present and lifetime psychopathology.

Finally, we did not assess the effects of alexithymia, a trait characterised by difficulties in identifying and describing emotions and impaired ability to distinguish bodily sensations from feelings (G. J. Taylor, Bagby, & Parker, 1991). Alexithymia is common in young people with ASD and is associated with higher levels of self- and parent-reported internalising problems in this population (Milosavljevic et al., 2015); therefore it could act as an additional aetiological factor in the development of mood and anxiety disorders in ASD youth. Furthermore, alexithymia, but not co-occurring ASD traits, may be related to some aspects of social reward processing (Foulkes, Bird, Gokcen, McCrory, & Viding, 2015) and interoception problems in adults (Bird et al., 2010; Shah, Hall, Catmur, & Bird, in press), suggesting that both ASD traits and alexithymia should be considered when investigating neurophysiological stress and reward responses. Nevertheless, at least in our physiology study, several factors argue against high levels of alexithymia in the ASD sample, including the very good parent-child agreement when reporting on irritability, similar item frequency patterns among boys with hfASD and SMD, and statistically equal subjective stress ratings between boys with hfASD and TD boys.

6.4 Clinical implications

As mentioned throughout this thesis, the research on comorbidity and its underlying mechanisms has direct implications for treatment. Not only can a delay in identifying a comorbid symptom affect the treatment outcomes for that symptom, but a therapeutic intervention for the primary disorder may be affected by the presence of a comorbidity. In this section I discuss how the results from this thesis may inform treatment for comorbid emotional symptoms in young people with ASD. In so doing, I expand on clinical implications already presented at the end of each individual chapter.

6.4.1 Measurement of symptoms

Recognition and accurate measurement of emotional symptoms is undoubtedly needed for their effective and targeted treatment. This may be of particular importance in youth with ASD, where some symptoms characteristic of anxiety and depression can be mistakenly dismissed as part of core ASD symptomatology and not given prompt clinical attention. In addition, over-reliance on parent- and observer-reports could lead to omission of important aspects of the child's psychopathology that are less overtly manifested (Magiati et al., 2014). To this end, our results suggest that boys with hfASD report reliably on their irritability symptoms, and that their reports of irritability do not correlate with anxiety, unlike the reports made by their parents. Clinicians may therefore benefit from obtaining both child- and parent-reports to gain a comprehensive view of the child's difficulties (Kaurin, Egloff, Stringaris, & Wessa, in press).

The clinical importance of measurement goes beyond accurate identification of the presenting symptomatology. Reliable and valid measurement tools are also indispensable for monitoring treatment progress. This concerns both traditional ways of testing treatment effectiveness, by using pre- and post-treatment measurement scales to estimate symptom severity, and alternative approaches such as neuroimaging in cases where the reliability of self-report is compromised. ASL appears particularly advantageous for treatment monitoring due to its very good test-retest reliability (Detre, Rao, Wang, Chen, & Wang, 2012; Hermes et al., 2007; Hodkinson et al., 2013), although the associated costs may prohibit its use in clinical practice. Lastly, accurate and full identification of *all* co-occurring mood problems is important for effective treatment, as undetected comorbidities may impede on treatment progress. For example, co-occurring depressive symptoms may reduce a young person's motivation to perform tasks as part of exposure treatment for anxiety.

6.4.2 Pharmacological treatments

As mentioned in the individual discussion section for the reward study (Section 4.5), our results suggest that both shared and distinct aetiological mechanisms may underlie the co-occurrence of ASD traits and anxiety, with some but not other neural activation patterns present in both main- and interaction-effects. If comorbid ASD and anxiety is indeed associated with distinct aetiological mechanisms, medications used to treat anxiety in the TD population may not be as effective in those with ASD_{ANX}, similarly to the aforementioned example of reduced methylphenidate effectiveness in $ADHD_{ANX}$ (Moshe et al., 2012; MTACooperativeGroup, 1999; Pliszka, 1989; E. Taylor et al., 1987). On the other hand, we also showed that participants with ASD_{ANX} display an enhanced anxiety-related response during reward processing, e.g. the relatively more reduced (than in the main effect of anxiety) lateral PFC and right caudate activation to negative feedback. This suggests that while some mechanisms of anxiety problems in ASD youth may resemble those of TD youth, they may manifest more intensely in $ASD_{ANX.}$ Consequently, while similar treatment options may be effective for anxiety in ASD and TD populations, changes to medication dosing may need to be considered.

6.4.3 Psychological treatments

Similar clinical implications to those pertaining to medication treatment may also apply to psychological interventions. For example, if the mechanisms underlying anxiety in youth with ASD are similar, but manifest more intensely than in TD youth, changes to psychological treatment intensity and length may need to be considered. Accordingly, RCT evidence for the effectiveness of adapted CBT approaches for anxiety in ASD youth is mounting, when compared against both a waitlist control (McNally Keehn, Lincoln, Brown, & Chavira, 2012; Wood et al., 2009) and a more active treatment-as-usual (Storch et al., 2013).

Second, while we did not fully distinguish between the effects of irritability and anxiety on stress responsiveness, irritability was associated with a dampened physiological response to stress, similar to what has been reported in TD youth with disruptive behaviour disorders (van Goozen et al., 2000; van Goozen et al., 1998). Dampened stress responsiveness has negative treatment implications in TD youth with ODD/CD, where those with lower cortisol responsiveness to a frustration task show reduced treatment gains following a course of individual CBT and parent management training (van de Wiel et al., 2004). Based on the frequent co-occurrence of irritability and anxiety in childhood (Stoddard et al., 2014), we could expect similar difficulties with psychological treatments for anxiety in cases where irritability is prominent. As

suggested by Foa and Kozak (1986), a sufficient amount of arousal may be needed in order to trigger a positive response to exposure-based treatments, frequently used for disorders such as OCD and social phobia. It remains to be tested whether the effects of irritability on reduced physiological responsiveness are similar in ASD and TD youth, or whether there is a further dampening of the physiological stress response in those with comorbid ASD and irritability, which could have a further impact on treatment effectiveness.

Third, if disrupted activity in the brain's emotional and attentional networks, as found in our reward processing study, does indeed underlie the co-occurrence of ASD and anxiety, treatment approaches based on fMRI neurofeedback may prove useful. For example, emerging evidence shows that real-time neurofeedback may lead to successful regulation of insula activity in adults with (Ruiz et al., 2013) and without psychopathology (Lawrence et al., 2013).

Finally, in parallel to investigating the mechanisms underlying emotional problems in ASD to guide effective treatments, future research should not overlook possible resilience factors that may protect young people with ASD from developing these problems in the first place. Emerging evidence suggests that ability to handle problems and good decision making act as protective factors against developing anxiety in boys with hfASD and may also reduce co-occurring irritability symptoms (Bitsika & Sharpley, 2014). Therefore, clinicians working with young people with ASD may benefit from introducing modules focused on problem solving strategies into their therapeutic interventions. Interestingly, some of the aforementioned resilience factors may depend on the functioning and plasticity of the brain's fear and reward circuits (Haglund, Nestadt, Cooper, Southwick, & Charney, 2007; Sonuga-Barke et al., 2016; van der Werff, van den Berg, Pannekoek, Elzinga, & van der Wee, 2013).

6.5 Overall conclusion

This thesis used a multi-method approach to address the gaps in existing literature concerning the measurement of and the mechanisms underlying mood and anxiety problems in young people with ASD. Based on the reviewed literature, it appears that the research on prevalence rates of emotional problems in ASD has not been paralleled with equivalent focus on the mechanisms underlying their comorbidity. We combined behavioural, physiological, and neuroimaging methodologies to investigate the effects of emotional symptoms on reward processing and stress responses in young people from community and clinical samples.

Our results suggest that some pathophysiological mechanisms of anxiety may indeed be different, or may manifest more intensely, in young people with combined ASD and anxiety. Future research will be needed to investigate whether atypical interoceptive prediction signalling and cognitive control mechanisms underlie these observed differences, as proposed in my

hypothetical model. Co-occurring irritability accounted for the effects of anxiety on cortisol stress responsiveness, but not on heart rate responsiveness or negative reward feedback. The effects of irritability on cortisol responsiveness show similarities to previous findings with TD youth. However, more research using dedicated irritability scales is needed to assess whether irritability presents differently in young people with vs. without ASD. Importantly, we showed that irritability can be measured reliably in the hfASD youth population.

We also explored alternative ways of measuring mood states in young people by using a technique that may help overcome the limitations of self- and informant-reporting in the future. While more research is required to pinpoint the rCBF patterns associated with low mood in ASD youth (and the methodological feasibility of such research efforts needs to be studied), ASL appears especially well-suited for examining the neural correlates of mood states relative to traditional BOLD fMRI, and our results suggest it is sensitive to experimentally-induced mood changes. Indeed, accurate and reliable measurement of symptoms is crucial to comorbidity research in ASD youth, both in terms of accurate estimation of prevalence rates and investigating the mechanisms underlying comorbidity. Table 6.1 (below) recommends avenues for future research based on the key results from this thesis.

In conclusion, our results suggest that both shared and distinct mechanisms may underlie the frequently-observed symptoms of anxiety and irritability in young people with ASD, compared to TD youth. Future research using validated measurement techniques in well-defined samples will be needed to further disentangle the complex interplay of anxiety, irritability, and core ASD difficulties. As suggested in this chapter, research into the pathophysiological pathways of comorbidity is likely to be most effective if conducted at multiple levels of explanation.

Table 6.1. Implications for further research based on the key results from this thesis.

Key result from this thesis	Outstanding questions and future research directions
Stress reactivity	
Irritability was associated with reduced cortisol stress reactivity in boys with ASD.	(1) Are the effects of irritability on physiological stress responsiveness similar in ASD and TD youth? (2) Alternatively, do young people with comorbid ASD and irritability display a further dampening of HPA axis reactivity to that observed in TD youth?
We did not fully distinguish between the effects of irritability and anxiety on stress responsiveness.	(1) How do physiological responses to stress compare between young people with ASD who display predominantly anxiety vs. predominantly irritability symptoms? (2) Could contextual factors (e.g., environmental contingencies) modulate fear- vs. anger-driven responses to stress?
Reward processing	
Young people with combined ASD traits and anxiety showed distinct hyperactivation in the right insula during reward anticipation.	(1) Do young people with ASD find the anticipation stage during reward tasks anxiety-provoking? Are they particularly susceptible to anticipating negative outcomes? (2) Are young people with ASD particularly susceptible to aberrant interoceptive prediction signalling? Does atypical interoceptive processing explain insula hyperactivation during reward anticipation?
Right-sided medFG and dACC activations during reward anticipation predicted future, new-onset anxiety in those with high ASD traits.	(1) Do ASD youth require additional attentional resources and/or enhanced conflict monitoring during reward anticipation to maintain on-task attention when faced with the competing effects of stressors? (2) Could psychological interventions focused on attentional bias modification prevent the development of anxiety in ASD youth?
Youth with combined ASD traits and anxiety showed the least activation in the lateral and medial PFC following negative reward feedback.	(1) Do these neural activation patterns represent ineffective cognitive control in stressful situations, such as during negative feedback? (2) Do top-down cognitive control deficits affect physiological stress response patterns in young people with ASD?
Alternative measurement	
Mood induction led to robust changes in self-reported mood ratings in healthy young people.	Is mood induction feasible in young people with ASD?

The effects of happy mood induction on rCBF patterns were generally stronger than the effects of sad mood.	(1) How long do rCBF changes generated by mood induction last? (2) Are the same rCBF patterns found if our experiment was replicated in a larger sample and with a randomised order of mood induction conditions?
Pattern recognition distinguished sad and happy moods from neutral, but not from each other, based on the associated whole-brain rCBF patterns.	(1) Are the effects of sad vs. happy mood induction easier to distinguish in young people with a history of depression? (2) Can functional connectivity analysis methods tell us more about the way in which different mood states affect neurophysiological response patterns?

ASD, autism spectrum disorder. *dACC*, dorsal anterior cingulate cortex. *HPA*, hypothalamic-pituitary-adrenal. *medFG*, medial frontal gyrus. *PFC*, prefrontal cortex. *rCBF*, regional cerebral blood flow. *TD*, typically developing.

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